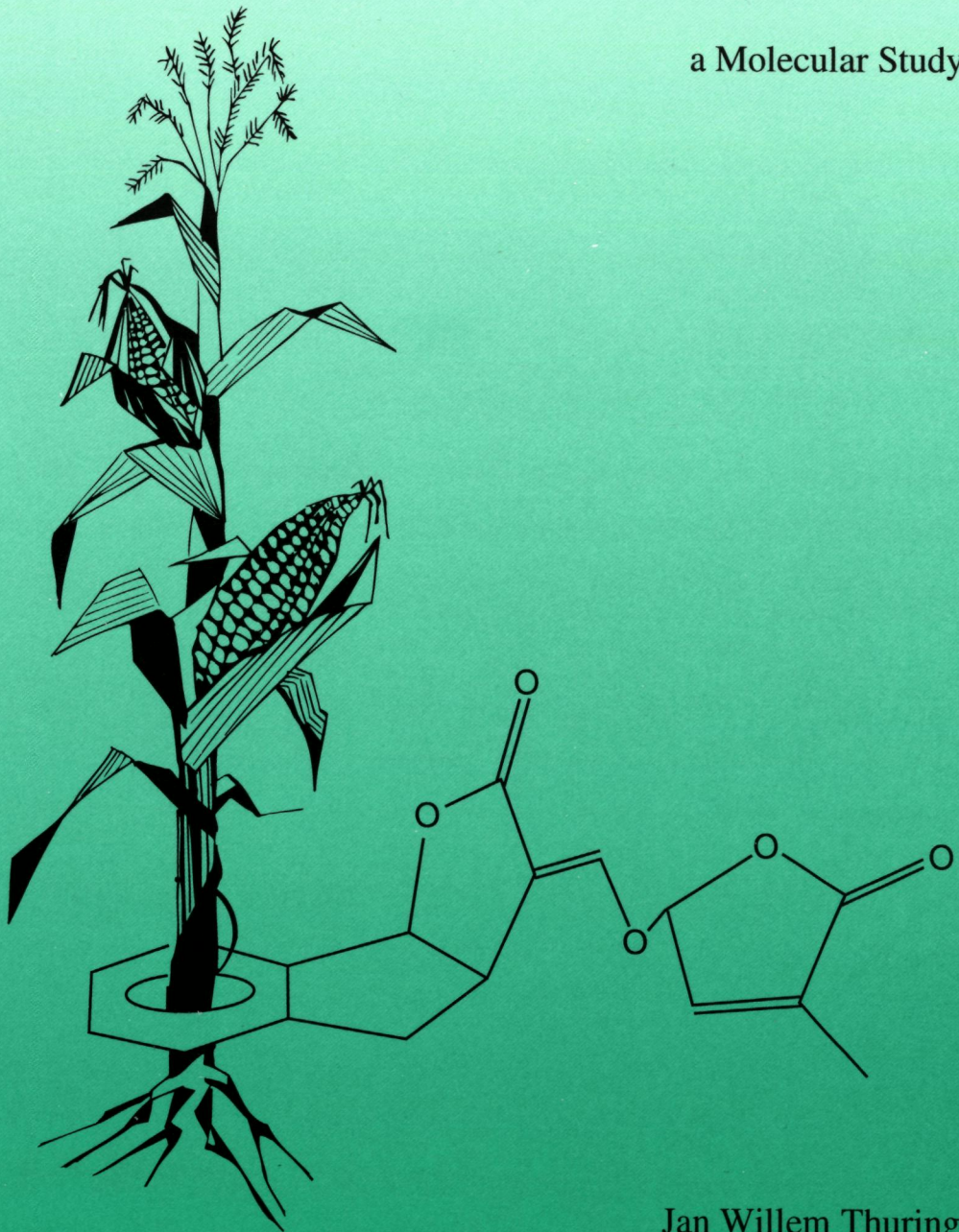
Synthesis and Bioactivity of Germination Stimulants for the Seeds of Parasitic Weeds

a Molecular Study



Jan Willem Thuring

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EEN WETENSCHAPPELIJKE PROEVE OP HET GEBIED VAN DE NATUURWETENSCHAPPEN

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE KATHOLIEKE UNIVERSITEIT NIJMEGEN, VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEDIGEN OP DONDERDAG 17 OKTOBER 1996 DES NAMIDDAGS OM 13.30 UUR PRECIES

DOOR
JOHANNES WILHELMUS JOHN FITZGERALD THURING
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Promotor:	Prof. Dr. B. Zwanenburg
Co-promotor:	Dr. G.H.L. Nefkens
Manuscriptcommissie:	Prof. Dr. U.K. Pandit (Universiteit van Amsterdam)
	Dr. A.F. Croes
	Dr. A.J.H. Klunder

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Aan mijn ouders

Paranimfen: Drs. N.W.J.T. Heinsman
Drs. M.A. Wegman

Voorwoord

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Jan Willem

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Chapter 1

Introduction

1.1 Background

The angiosperms *Striga* and *Alectra* (Scrophulariaceae), and *Orobanche* (Orobanchaceae) are root parasitic plants, which can only survive when attached to the roots of an appropriate host plant. As a consequence, these parasitic weeds may have an extremely devastating impact on their hosts. Important members of the first-mentioned family of parasites include *Striga hermonthica* (Del.) Benth. and *Striga asiatica* (L.) Kuntze, which attack preferentially monocotyledonous crops like maize, sorghum, millet and rice in tropical and sub-tropical areas in Africa and Asia (especially India). Another important species, *viz. Striga gesnerioides* (Willd.) Vatke, mainly parasitizes the legume crop cowpea and is widely distributed in Africa. The most common *Orobanche* species, *viz. Orobanche crenata* Forsk. and *Orobanche aegyptiaca* Pers., predominantly occur in the Mediterranean area, eastern Europe and in the Middle East. *Orobanche crenata* has a rather narrow host-range, from which faba bean is the most seriously affected, whereas *Orobanche aegyptiaca* has a wide host-range, including several dicotyledonous crops like tomato, tobacco and sunflower. Due to their enormous impact on agriculturally important crops, the occurrence of these parasitic weeds directly affects the lives of millions of people in the areas mentioned above. The seeds of these weeds are produced in vast amounts (up to 100,000 per plant), are tiny (< 0.5 mm in diameter) and remain viable in the soil for long periods (up to 20 years). These factors make the control of these weed pests extremely complicated and therefore several eradication programs have only been partially successful. In fact, the *Striga* (witchweed) problem seems even to get worse due to intensive monocropping of improved cultivars, which are however often highly susceptible to *Striga*.

This introductory chapter describes only very briefly some relevant aspects of the life cycle of the parasite, the stimulation of seed germination, and possible control strategies. Most information is abstracted from recent (book)reviews, which in addition cover several other aspects regarding parasitic weed species and the interactions with their hosts.[1-10]

1.2 Life cycle of root parasitic weeds

The lifestyle of the root parasitic weeds *Striga* and *Orobanche* is extremely well adapted to that of their host plants, which does not only involve dependence for water, minerals and energy,

but also the perception of several developmental signals at various stages in their life cycle, which reach down to the molecular level. Because the holoparasite *Orobanche* completely lacks chlorophyll and the obligate hemiparasite *Striga* can only partly assimilate, they are entirely dependent on their host to survive and to develop. As soon as the parasitic seeds are shed they undergo a period of after-ripening, which may last up to 6 months. During this time the seeds are dormant and hence not capable to germinate. The seeds require a so-called conditioning period, which involves exposure to a warm moist environment at tempeartures ranging from 20-35^{0}C for 7-14 days before they acquire the ability to germinate. At present it is not completely understood which factors break dormancy. The removal of germination inhibitors from the parasitic seed during conditioning, weakening of the seed coat and/or induction of enzymes, which are responsible for the biosynthesis of the plant hormone ethene, have been proposed as important determinants to break dormancy. Non-dormant seeds of *Striga* and *Orobanche* will only germinate when they receive an external chemical trigger, *i.e.* a germination stimulant, which is present in the root exudate of a potential host. Following the stimulation of germination the parasitic seed develops a radicle, which rapidly elongates to make contact with the host root. Then the development of a specialized organ, *i.e.* the haustorium, starts, which is initiated by other host-derived chemical substances, *e.g.* 2,6-dimethoxy-*p*-benzoquinone. The parasite becomes firmly attached to the surface of the host root by means of this haustorium, which may be regarded as a physiological and morphological bridge between host and parasite. After penetration the integration with the host vascular system starts competition for host resources, which accounts to some extent for its reduced development. It should be noted that also attachment and penetration require host-derived chemical stimuli, the structures of which are yet unknown.

1.3 Germination stimulants

The first naturally occurring germination stimulant identified is (+)-strigol (fig. 1). This compound was isolated in 1966 from the root exudate of cotton (*Gossypium hirsutum* L.),[11] which however is not a host for *Striga* and *Orobanche*. The relative configuration of (+)-strigol was determined in 1972,[12] while the correct absolute stereochemistry was established unambigiously in 1985 by means of an X-ray diffraction analysis.[13] Its activity as a seed germination stimulant toward a variety of *Striga* species is very high, half-maximal effects at concentrations as low as 10^{-10}M have been reported.[14-16] Seeds of *Orobanche* species are generally less responsive to (+)-strigol, *viz.* at concentrations of 10^{-7}-10^{-8}M.[17] Following its structure elucidation, several total and partial syntheses of strigol have been reported.[18-36]

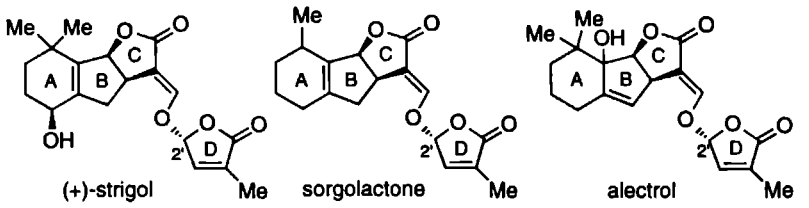


Figure 1

Because strigol was isolated from a non-host, its significance in the true host-parasite interaction was uncertain for a long time. A breakthrough was achieved when Hauck *et al.* proposed sorgolactone (fig. 1), a close analog of strigol, as the major *Striga* germination stimulant produced by sorghum roots.[15] Approximately at the same time these authors reported that another strigol analog, *viz.* alectrol (fig. 1), is produced by cowpea roots and which has a high potency in the stimulation of seed germination of *Striga gesnerioides*.[16] Soon thereafter Butler and coworkers showed that strigol itself is the major germination stimulant produced by the *Striga* hosts maize and proso millet.[37] For this class of compounds (fig. 1) the collective name "strigolactones" has been introduced.[9] In addition it has been demonstrated that root exudates of *Striga* hosts generally contain a mixture of these strigolactones, albeit in different ratios.[37] Until now a total synthesis of sorgolactone or alectrol has not been reported; only some partial syntheses of sorgolactone are known.[34,36] So far, germination stimulants produced by *Orobanche* hosts have not been identified.

Substitute germination stimulants

Apart from the strigolactones and synthetic strigol analogs (*vide infra*), several other compounds are known to stimulate seed germination of root parasitic angiosperms. Before the identification of the strigolactones (fig. 1) as the major stimulatory constituents produced by *Striga* hosts, dihydrosorgoleone (fig. 2) has been claimed as the actual stimulant exuded by a true *Striga* host, *viz.* sorghum.[38] This highly unstable compound is several orders of magnitude less potent than strigol. Subsequent studies have disproved this hypothesis, as there is no correlation between the amount of dihydrosorgoleone produced by several genotypes of sorghum and their susceptibility to *Striga*,[39] whereas this correlation does exist for the strigolactones.[37]

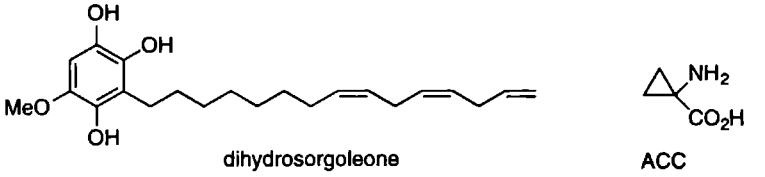


Figure 2

It has been shown that the plant growth regulator ethene is able to trigger seed germination of a limited number of parasitic weed species, *viz. Striga asiatica*[40] and *Striga hermonthica*,[41] in the absence of other chemical stimuli. In addition, 1-aminocyclopropane-1-carboxylic acid (ACC, fig. 2), the precursor of the biosynthesis of ethene, also causes germination of some *Striga* species.[5] It has been suggested that strigol and its analogs may exert their effect by eliciting the biosynthesis of ethene, based on observations that seeds of *Striga asiatica* and *Striga hermonthica* generate ethene when treated with strigol(analogs).[42-45] It is worth noting that also some oxidizing agents such as sodium hypochlorite and sulfuric acid stimulate germination, possibly as a consequence of the chemical oxidation of endogenous ACC to ethene.[5]

Several other plant growth regulators such as cytokinins, *e.g.* dithiazuron,[46] gibberellins and auxins have been reported to stimulate *Striga* and *Orobanche* seed germination. These effects were observed either in the absence of or in combination with host-derived stimuli to increase germination when applied together with host root exudate.[5]

It is important to note that generally these alternative germination stimulants exert their effect at much higher concentrations than the strigolactones, and thus have a much less specific mode of action.

1.4 Control strategies[6]

An attractive approach for the control of parasitic weed pests is interfering in the intimate relation between host plant and parasite. Thus, the stimulation of germination in the absence of a host plant will kill the germinated seeds and hence reduce the seed population in the soil. This concept of "suicidal germination"[47] may be applied either by biological or chemical means. The first-mentioned option involves the use of trap-crops, *i.e.* plants which produce germination stimulating substances but are not susceptible to attack, or catch-crops, *i.e.* normal hosts which stimulate germination and can carry the parasite to full development, but require destruction before any parasite seeds are formed. One of the drawbacks of these biological methods is that the reduction of seed population is rather limited, because the active components in the root exudates are only active within a short distance from the host roots. As a chemical alternative, the injection of ethene in infested fields in some parts of the USA has resulted in high depletion of the seed bank (*Striga asiatica*).[48] However, this technology is very expensive and only applicable to parasitic weed species that are responsive to ethene. The use of strigol itself as a suicidal germination agent is not feasible, as its synthesis is too complicated and its stability in the soil is rather limited to become practically applicable.[49,50] In order to circumvent these problems the access to relatively simple synthetic strigol analogs possessing high stimulatory activities and better stability properties is required (*vide infra*). It should be emphasized here that instability problems can also be overcome by using an appropriate formulation of the stimulant.

Other control methods include hand-pulling of the weed before seed production, the use of nitrogen fertilizers, breeding of resistant and tolerant crop varieties, and herbicides which kill the parasitic plants either before or after emergence. For an overview about the prospects and limitations of these strategies, see ref. 6.

1.5 Synthetic strigol analogs

For reasons outlined above, great effort has been put in the preparation and testing of analogs of strigol after its structure elucidation. An important issue of these studies was the identification of the bioactiphore, *i.e.* the part of the molecule which is primary responsible for the biological activity. Insight in this essential part of the molecule would lead to a more rational design of simple, highly active analogs, which might be suitable as herbicides to eradicate these parasitic weed pests. The approaches that have been considered can be divided into three classes, which all have literature precedents.

i. *Simplification of the strigol molecule by leaving the main frame work intact.* The overall characteristic of these analogs is retention of the ring system of strigol, *i.e.* the ABCD-skeleton (fig. 3). Some examples of this approach are, among others, the stereoisomers of natural (+)-strigol, which exhibit quite distinct activities. As expected the "naturally" configurational isomer is the most active one for *Striga*[14] and Orobanche,[17] whereas its optical antipode exerts a more pronounced effect on the related root parasite *Alectra*.[14] Removal of the hydroxy group resulting in racemic 5-deoxy strigol **1** (fig. 3) did not lead to considerable loss of activity.[17]

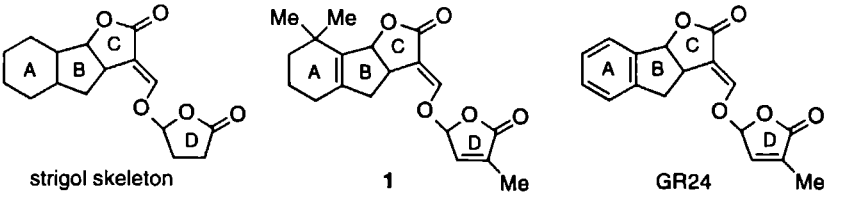
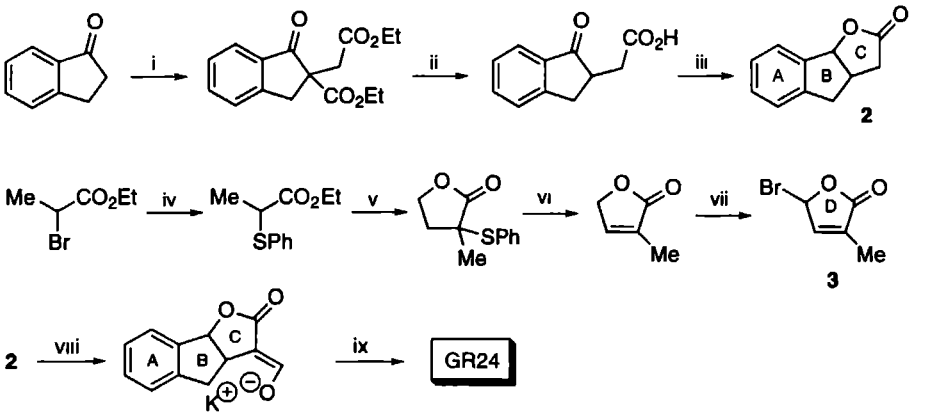


Figure 3

A very important modification was the replacement of the cyclohexene A-ring by an aromatic ring to give GR24 (fig. 3), the synthesis of which is much simpler than that of strigol, as was first reported by Johnson *et al.* in 1981.[51] Several years later its preparation was considerably improved, and the separation of its diastereomers was achieved.[52] The synthesis of GR24 as is outlined in scheme 1 represents a general tactic for the preparation of strigol and its structural analogs. An appropriately α-formylated ABC-precursor is O-alkylated with a butenolide-type D-ring precursor, *e.g.* **3**, containing a leaving group at the γ-position to give the desired product in a completely *E*-selective fashion;[20,21] the formation of the *E*-isomers arises from a

thermodynamical preference.[21,53] Bioassays revealed that the activity of GR24 is comparable to that of strigol, *c.f.* refs. 15, 17, 52.

Scheme 1



i. a. $(EtO)_2CO$, NaH, DMF; b. $BrCH_2COOEt$. ii. 6N HCl(aq.), HOAc, reflux.
iii. a. $NaBH_4$, 0.2N NaOH(aq.); b. pTosOH, C_6H_6, reflux.
iv. PhSNa, EtOH. v. a. LDA, THF; b. oxirane; c. H^+. vi. a. $NaIO_4$, $MeOH/H_2O$; b. CCl_4, Δ. vii. NBS
viii. a. KOtBu, HCO_2Me, in THF. ix. a. bromobutenolide **3**. b. separation of diastereomers

ii. *Systematical removal of parts of the strigol skeleton.* This type of modification has attracted much attention and only some relevant examples will be discussed. A major contribution came from the group of Johnson, who prepared a series of so-called GR-compounds, *e.g.* BCD-analogs GR28 and GR7, CD-analog GR5 and D-ring analog **4a** (fig. 4).[51,54] Analogs GR24, GR28, GR7 and GR5 all possess considerable germination stimulatory activity, although it gradually decreases in the indicated order.[17,52,54,55]

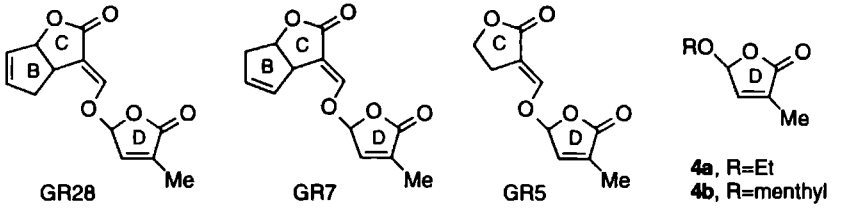
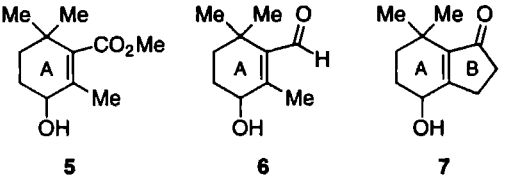


Figure 4

In contrast, γ-ethoxy butenolide **4a** is completely inactive in *Striga* seed germination,[54] the same result was obtained for γ-menthyloxy derivative **4b** on *Orobanche crenata*.[17] However, Pepperman *et al.* reported a considerable activity for **4a** as a witchweed (*Striga*) germination

stimulant, whereas several other γ-alkoxy butenolides were inactive.[56,57] This anomalous result is due to the bioassay procedure used (*vide infra*).

Several A- and AB-analogs have been prepared and biologically evaluated,[56,58] *e.g.* compounds **5** and **6** (fig. 5). It was concluded that most of these analogs were active. However, a re-evaluation of these results was performed by testing compounds **5-7** and some other AB-analogs. All these compounds were completely inactive in these bioassays,[59] which implies that the original claim is incorrect.

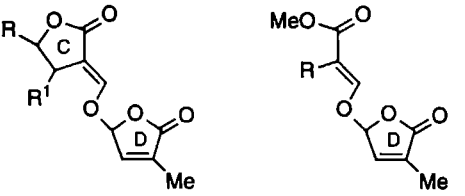


5 **6** **7**

Figure 5

Inconsistencies in the bioactivities of strigol analogs are thus frequently encountered in the literature, which must be attributed to differences in the respective bioassays. In order to avoid these problems, a standardized bioassay has been described in detail.[60] Amongst others, one essential feature is the use of acetone as a co-solvent to prepare the aqueous stimulant solutions, and not dimethyl sulfoxide (DMSO) as was used by Pepperman *et al.*[56-58] DMSO may cause oxidation of ACC to ethene (*vide supra*), which may exert synergistic effects with the actual stimulant. Another important aspect is to include GR24 as a positive control in the bioassay to which other results can be related, and thus eliminates problems due to variable seed responses in different test series.

Evaluation of the stimulatory activity of strigol analogs with modifications in the tricyclic ABC-fragment, *e.g.* compounds **8-13** (fig. 6),[61] revealed that this part is not essential to retain biological activity. Interestingly, ACD-ring analog **8** is almost as active as GR24, whereas its synthesis is far less complicated.



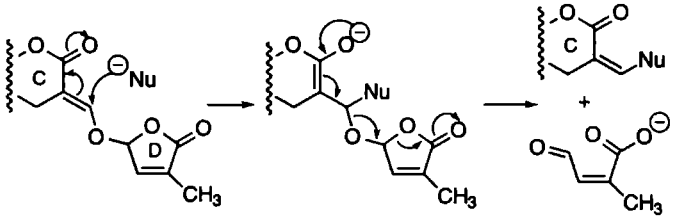
8, R=Ph, R^1=H
9, R=H, R^1=Ph

10, R=indanyl
11, R=CH_2CH_2Ph
12, R=CH_2Ph
13, R=Ph

Figure 6

iii. *Modifications based on a possible molecular mechanism for the induction of germination.* The bioactivity of strigol and its analogs has been analyzed in terms of a structure-activity relationship. The observed bioactivity was primarily attributed to the α,β-unsaturated vinyl ether linkage and the D-ring. This has led to the proposal of a molecular mechanism as an initial trigger of the stimulation of germination, which is depicted in scheme 2.[62]

Scheme 2



This molecular mechanism involves addition of a nucleophilic species, present at the receptor site, in a Michael fashion, followed by elimination of the D-ring. The ultimate result is that the ABC-part of the stimulant is covalently bonded to the receptor, a chemical change that may be reponsible for triggering germination. Some evidence for the validity of this mechanism has been acquired. Replacing the vinyl bond by a single bond to give **14** (fig. 7) led to complete loss of activity.[62] In addition, the addition-elimination reaction initiated by the receptor was mimicked by the reaction of GR24 with some nucleophiles, such as benzenethiolate, to give the expected product **15**.[62]

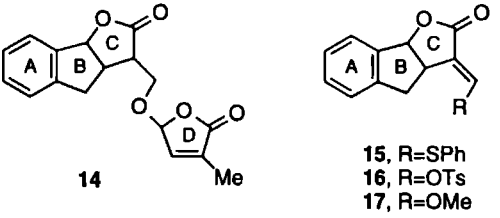


Figure 7

However, replacement of the butenolide D-ring by other leaving groups, such as a tosylate as in **16**[62] or methoxy group as in **17**[17] led to complete loss of activity. These results indicate that the D-ring does not only fulfil a role as a leaving group, but should meet (critical) spatial requirements as well. As a matter of fact, accurate data regarding these structural features are very scarce.[63]

1.6 Aim and outline of the thesis

The aim of the research described in this thesis is to gain more insight in the effects of (subtle) chemical modifications in the strigol skeleton on the seed germination stimulatory activity toward the parasitic weed species *Striga* and *Orobanche*.

In chapter 1 the background of the research is described together with an overview of the results of related preliminary studies. Chapters 2-4 deal with synthetic approaches for the introduction of optical activity in the butenolide D-ring. In chapters 5 and 6 applications of this newly devised methodology are described and the effects of the absolute stereochemistry on the biological activity are discussed. Chapter 7 is devoted to the synthesis and biological activity of a novel ABD-ring strigol analog. In chapter 8 a new and efficient procedure for the preparation of a precursor of ring D for strigol and its analogs is described. Chapter 9 deals with the influence of D-ring modifications on the biological activity. Chapters 10 and 11 are devoted to structural modifications in the vinyl ether linkage between rings C and D; in the former the reactivity of this moiety is affected by replacing the oxygen atom by a methylene function, whereas in the latter its intrinsic reactivity is retained, but its chemical environment is altered. Chapter 12 deals with synthetic approaches for the introduction of labeled substituents in the A-ring with the aim to obtain suitable substrates for the identification of the strigol receptor. Chapter 13 is devoted to the relation between ethene generation and germination. Possible interactions of GR24 in the biosynthesis of ethene and/or its subsequent effects are discussed. Chapter 14 describes synthetic approaches to analogs of aminoethoxyvinyl glycine (AVG), which are potential inhibitors of the biosynthesis of ethene. This thesis is concluded with summaries in English and Dutch.

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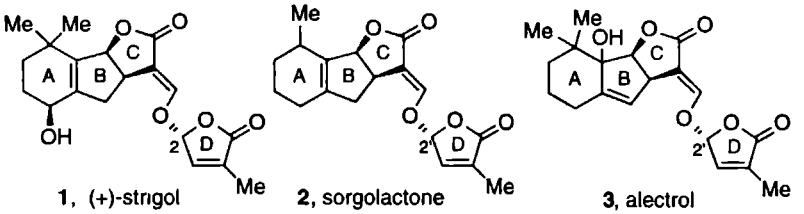
Chapter 2*

Asymmetric Synthesis of a D-ring synthon for Strigol Analogs and its Application to the Synthesis of all Four Stereoisomers of Germination Stimulant GR7

Abstract: A novel asymmetric synthesis of the strigol analog GR7 has been developed. The olefinic double bond of the butenolide D-ring was protected as a Diels-Alder adduct with cyclopentadiene The thus obtained tricyclic compound was resolved and transformed into a suitable D-ring synthon. The coupling reaction with hydroxymethylenolactone, which is the GR7-precursor, proceeded with complete stereocontrol. Cycloreversion under relatively mild conditions gave GR7 in an enantiomerically pure form.

2.1 Introduction

The germination of the seeds of the parasitic weeds *Striga*, *Alectra*, and *Orobanche* has received much attention, especially the compounds by which this process is induced.[1,2] Germination of the seeds of these parasitic weeds is triggered by a chemical species exuded by roots of a suitable host plant (+)-Strigol **1** was the first isolated naturally occurring germination stimulant from the root exudate of the false host cotton (*Gossypium hirsutum* L) and its structure was elucidated by Cook.[3] The absolute configuration was unambigiously determined by Brooks several years later.[4] Only very recently strigol has also been found in the root exudates of *Striga* host plants.[5] Some structures closely related to strigol (sorgolactone **2** and alectrol **3**) have been proposed for stimulants occurring in the root exudates of *Sorghum bicolor* and cowpea (*Vigna unguiculata*), which are hosts for *Striga* and *Alectra* species, respectively.[6,7]



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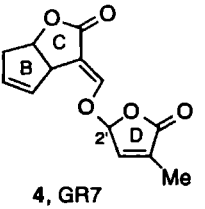
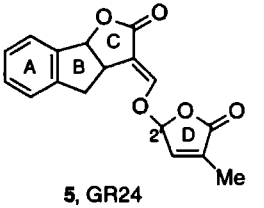


4, GR7



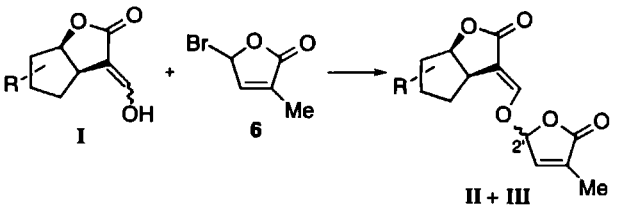
5, GR24

An attractive way for parasitic weed control is to use these germinating agents as herbicides in the absence of suitable host plants (concept of suicidal germination).[8] However, these naturally occurring germination stimulants are not suitable for this purpose, due to their complicated structures and to their intrinsic lability in alkaline soils. Inspired by the work of Johnson,[9] who prepared highly potent strigol analogs **4** and **5**, commonly known as GR7 and GR24, respectively, and that of Pepperman,[10] we have synthesized several structurally simpler biologically still active analogs of (+)-strigol with the aim to overcome these problems.[11,12,13,14]

Thus far, relatively scarce attention has been paid to the influence of the stereochemistry on the activity of strigol analogs. This is mainly due to the fact that no general method is known for the synthesis of homochiral strigol analogs. Optically active strigol has been obtained by resolution of racemic strigol,[15] resolution of the ABC-part of strigol,[4,16,17] and by asymmetric synthesis (chiral pool approach).[18] From all synthetic strigol analogs synthesized so far, only GR7 and GR24 have been obtained in an enantiopure form. All four stereoisomers of GR7 were prepared by starting from either of the two commercially available optical antipodes of Corey's lactone.[19] A similar tactic was followed in the synthesis the stereoisomers of GR24 as is detailed in chapter 5. From appropriate bioassays it was concluded that the stereochemistry at C-2' is essential to retain full germination stimulatory activity.[19,20] This finding was confirmed by Welzel in a study in which the seeds of *Orobanche crenata* were treated with all stereoisomers of strigol.[21]

Thus far, homochiral strigol and some analogs have been obtained starting from an enantiopure ABC-precursor **I**, which upon coupling with racemic bromobutenolide **6** and separation of the thus obtained diastereomers, affords the corresponding homochiral strigol analogs **II** and **III** (scheme 1).

Scheme 1



It should be more beneficial to control the stereochemistry at C-2' for the following reasons:

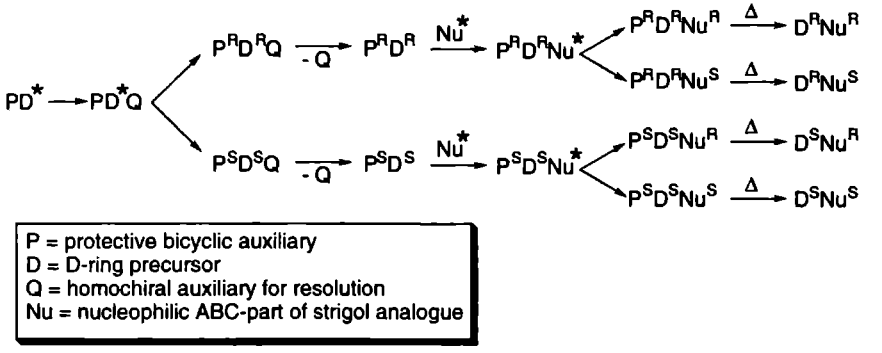
i The germination activity is highly sensitive to structural modifications in the D-ring.[22] This means that the D-ring is a common structural feature of biologically active strigol analogs.
ii The configuration at C-2' is essential for a high biological activity (*vide supra*).
iii Control of the stereochemistry at C-2' will enable the synthesis of homochiral strigol analogs, which are achiral in the ABC-part.

In this chapter a novel, versatile synthetic route to homochiral strigol analogs with complete stereocontrol at C-2' is presented.

2.2 Results and discussion

In order to achieve stereocontrol at C-2' it is essential to protect the double bond in **6**. While our investigations were in progress, Welzel[23] published a strategy, involving a phenylsulfanyl group as double bond protection and to control stereoselective bond formation at C-2'. However, this method is rather laborious and needs considerable improvement. Conceptually, our approach is outlined in figure 1.

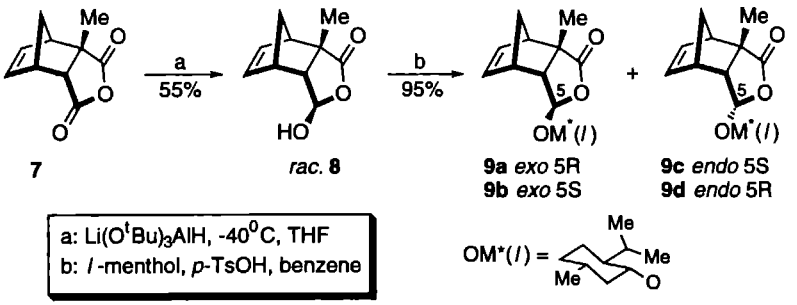
Figure 1



Protection of the double bond of the D-ring as a Diels-Alder adduct gives racemic PD*, which is subsequently resolved by an enantiopure auxiliary Q. A suitable racemic ABC-synthon of a strigol analog is coupled to the thus obtained enantiomers of PD. Separation of the diastereomers, followed by removal of the auxiliary P affords all possible stereoisomers of the strigol analog.

As is depicted in scheme 2 the starting material in the Diels-Alder adduct of citraconic anhydride and cyclopentadiene **7**, which has been used previously in the synthesis of the racemic butenolide **6**.[24]

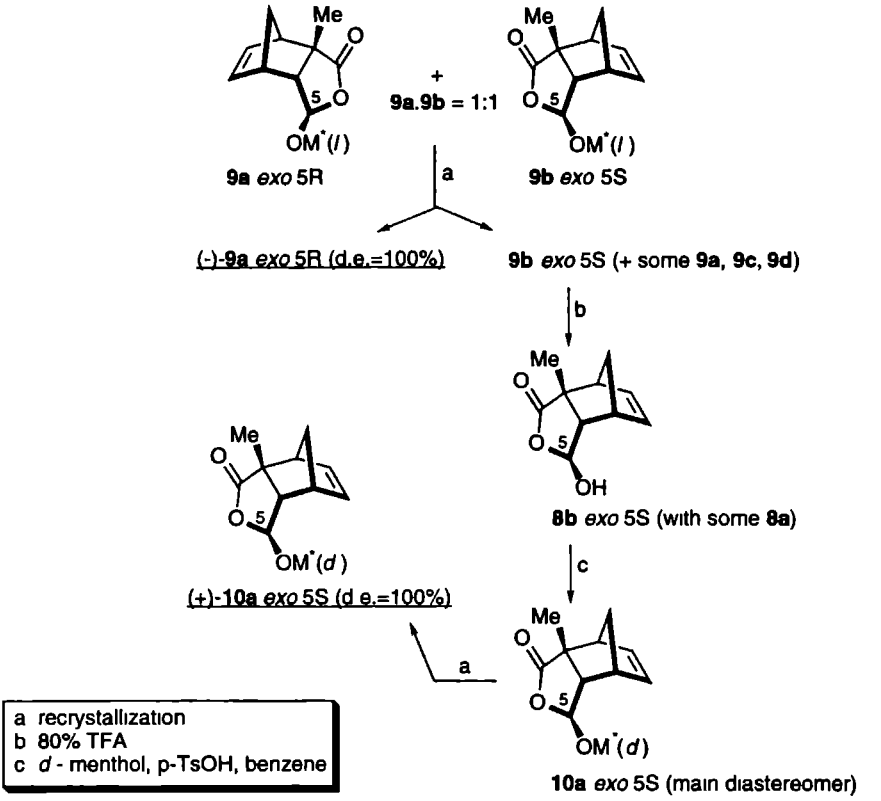
Scheme 2



Partial reduction employing $Li(O^tBu)_3AlH$ gave hydroxy lactone *rac.* **8**.[25] This transformation occurred with complete diastereoselectivity; only the *exo*-hydroxy epimers were obtained. At this stage it is appropriate to perform the resolution. Treatment of *rac.* **8** with *l*-menthol in the presence of a catalytic amount of *p*-TsOH under azeotropic conditions for 18 h gave a mixture of *exo*-5R-, *exo*-5S-, *endo*-5R-, and *endo*-5S-*l*-menthyloxy lactones in a ratio of 44:44:6:6. When the reaction was stopped after 4 h the product distribution of **9(a+b)**:**9(c+d)**:**8** amounted to 52:24:22, suggesting that the initially formed *endo*-isomers **9(c+d)** (kinetic products) epimerize under the reaction conditions to the thermodynamically more stable *exo*-isomers **9(a+b)**. The product distribution could unambigiously be determined by an ^{1}H-NMR analysis. The *exo*/*endo* assignments were made on the basis of chemical shifts and coupling constants. The acetal proton H_5 of the *endo*-isomers **9(c+d)** exhibited a doublet (^3J = 6.7 Hz) at ca. 0.7 ppm lower field as compared to the corresponding *exo*-isomers **9(a+b)** (^3J = 1.2 Hz). The coupling constants were verified by MM-2 calculations and are in complete agreement with those reported for similar systems.[26] Diastereoisomer (-)-**9a** has already been synthesized by Feringa[27] via a different route, although no analytical data were reported. Without any further purification diastereomer (-)-**9a** could be crystallized selectively from the crude reaction mixture (from *n*-hexane, 100% d.e., 28% yield). It was not possible to obtain more of this diastereoisomer in a pure form by repeated crystallization of the residue. Therefore, the starting hydroxy lactone **8** (enantiomerically enriched) was recovered and treated as is outlined in scheme 3.

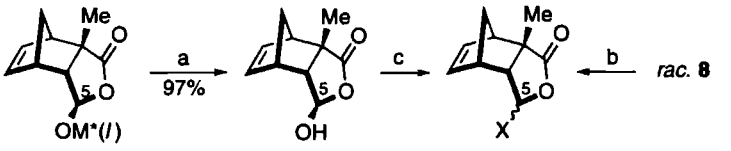
The residue, containing mainly **9b** and smaller amounts of **9a**,**9c**, and **9d** was hydrolyzed in 80% TFA to give **8b** (enantiopurity ≈ 69%), which could readily be purified by a quick filtration over silica. Subsequent treatment with *d*-menthol under azeotropic conditions gave (+)-**10a** as the main stereoisomer, which is the enantiomer of (-)-**9a** and could thus again readily be crystallized from the crude mixture (24% yield, 100% d.e.). This procedure is easy to perform and can be accomplished without significant loss of material. With both enantiopure menthyloxy lactones (-)-**9a** and (+)-**10a** in hand, these were transformed into suitable synthons for coupling reactions with strigol precursors of type I (scheme 1). The chiral auxiliary *l*-menthol was readily removed by hydrolysis in 80% TFA leading to enantiopure hydroxy lactone (+)-**8a** (scheme 4).

Scheme 3



In order to substitute the hydroxyl function by a halogen atom, some test experiments were performed starting from *rac.* **8**. Bromination under S_N2 conditions (CBr_4, PPh_3, Et_3N) of racemic **8** gave after 18h a mixture of two isomeric products (*exo*-**11** and *endo*-**12**) in a ratio 1:1 Careful TLC analysis revealed that initially *endo*-**12** was formed as the kinetic product, which slowly epimerized to *exo*-**11** This epimerization can be envisaged by assuming the formation of an oxonium ion *via* elimination of bromide, followed by trapping this intermediate from either the *endo*- or the *exo*-face. Unfortunately, *exo*-**11** and *endo*-**12** are unstable and, in addition, they did not give satisfactory results in the coupling reactions Therefore, the synthesis of the corresponding chloro lactone (-)-**13a** was undertaken. Treatment of enantiopure (+)-**8a** with excess $SOCl_2$ in the presence of 1 equivalent of pyridine smoothly gave both epimers *exo*-(-)-**13a** and *endo*-(-)-**14a** in a ratio of 6:1 in almost quantitative yield. The same result was obtained in the absence of pyridine as the HCl-scavenger. Again, *endo*/*exo* assignments were made on the basis of coupling constant (1.0 vs. 7.0 Hz) and the difference in chemical shift. This diastereomeric mixture was separated by column chromatography to give enantiopure *exo*-(-)-**13a**.

Scheme 4



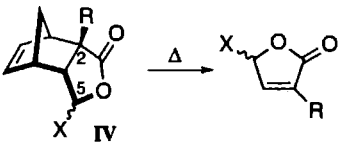
9a (5R) **8a** (5R) X=Br, *exo rac.***11**:*endo rac.***12** = 1:1
X=Cl, (-)-*exo* **13a** (5S): (-)-*endo* **14a** (5R) = 6:1

a: 80% TFA, 15 h.
b: CBr_4, PPh_3, Et_3N, 18 h., c.y.= 70%
c: $SOCl_2$ (xs.), pyridine, 1 h., c.y.= 91%

The coupling reaction of (-)-*exo*-**13a** with the GR7 precursor, *i.e. rac.* hydroxymethylenolactone **15**,[19] gave two diastereomeric adducts **16a** and **16b** in the expected ratio of 1:1 with complete *exo*-selectivity (scheme 5). It should be noted that the R/S-assignment in (-)-**13a** and **16** has changed, due to the priority rules. Starting from (+)-*exo*-**13b**, the corresponding enantiomers **16c** and **16d** could be synthesized in the same manner. Both diastereomeric pairs, *viz.* **16a,b** and **16c,d**, were separated by flash chromatography. The substitution reaction thus proceeds with retention of configuration. This observation suggests that the reaction takes place *via* an oxonium ion, which is subsequently trapped by the enolate, derived from *rac.* **15**, from the *exo*-face.

Finally, the cycloreversion step was investigated. In the literature only three reports are known in which a system of type IV, having an alkyl substituent at C-2, is subjected to a retro Diels-Alder reaction (scheme 6):

Scheme 6



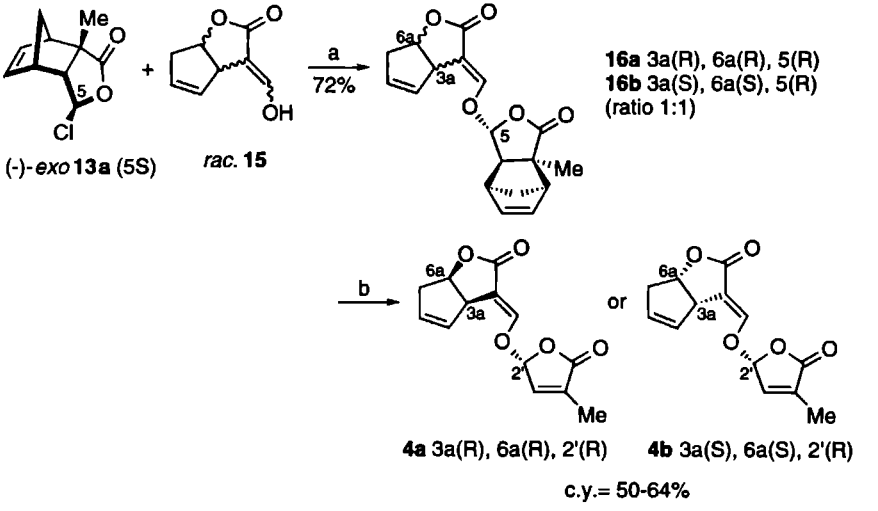
The reaction conditions are either heating at 240^{0}C-285^{0}C for several days in a sealed tube[28] or thermolysis under flash vacuum conditions (short contact time) at 300^{0}C-330^{0}C[29] or at 500^{0}C (X = H).[24]

In order to prevent epimerization at C-2' in **4** during the thermolysis, the reaction should be carried out under mild conditions. This could be accomplished by heating the Diels-Alder adducts **16a**, **16b** and **16c**, **16d** in *o*-dichlorobenzene at 180^{0}C for 15h (scheme 5). Under these conditions the cycloreversion occurred without significant epimerization in yields of 50-64%. In this manner all four stereomers of GR7, *viz.* **4a**, **4b**, **4c** & **4d** were obtained in an enantiopure form. The physical data are in complete agreement with those previously reported.[19] It is noteworthy that

heating of the *l*-menthyloxy lactone (-)-**9a** under the same conditions led to the corresponding butenolide, which was completely epimerized at C-5.

In conclusion, a highly efficient route with excellent stereocontrol is developed for the synthesis of all stereoisomers of the synthetic strigol analog GR7. In principle, this method can easily be extended to the asymmetric synthesis of other strigol analogs. This aspect, along with the optimalization of the resolution step is discussed in chapters 5-7 and chapters 3-4, respectively.

Scheme 5



2.3 Experimental section

General remarks

100 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard) and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me_4Si as internal standard). All coupling constants are given as ^3J in Hz, unless indicated otherwise For mass spectra a double focussing VG7070E mass spectrometer was used GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30m x 0.25 mm). Helium was used as carrier gas, and electron impact (EI) was used as ionization mode.

GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas Melting points were measured with a Reichert Thermopan microscope and are uncorrected Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.

Solvents were dried using the following methods: Dimethylformamide (DMF) p a was dried on 4Å molecular sieves. Dichloromethane was distilled from P_2O_5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH_2. Ethyl acetate was distilled from K_2CO_3. Trifluoroacetic acid (TFA) was used as an 80% (v/v) aqueous solution All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0 25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray "Flash" chromatography was carried out at a pressure of *ca.* 1.5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was carried out, using Merck Kieselgel 60.

5(R)-[2(S)-Isopropyl-5(R)-methyl-(R)-cyclohexyloxy]-2(S)-methyl-4-oxa-endo-tricyclo[5 2 1.0^{2,6}]dec-8-en-3-one (**9a**) *and its enantiomer* (**10a**)

Rac *exo*-hydroxy tricyclic lactone **8**[25] (7.60 g, 42.2 mmol) and *l*-menthol (7.90 g, 50.7 mmol) were dissolved in benzene (125 mL) containing 0.05 eq. *p*-TsOH (401 mg, 2.11 mmol). The mixture was heated under reflux for 18h, using a Dean-Stark trap. After evaporation of the solvent, the residue was dissolved in a mixture of saturated $NaHCO_3$ and ethyl acetate Extraction with ethyl acetate (2x), washing the combined organic layers with brine, and drying ($MgSO_4$) provided crude product in quantitative yield. Based on ^{1}H-NMR analysis the product consisted of a mixture of 4 diastereomers **9a-d** in a ratio 44:44:6·6 The crude mixture was crystallized from *n*-hexane to give pure **9a** (3.72 g, 28%) as colorless needles. Mp 131.5-132 5 ^{0}C; $[\alpha]_D$ -147^0 (c 0.40, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$. 100 MHz). δ 0.72-1.02 (m, 12H), 1.20 (m, 3H), 1.52 (s, 3H), 1 64 (m, 3H), 2 01-2 18 (m, 2H), 2.45 (dd, J = 0.9, 4.1 Hz, 1H), 2.82 (m, 1H), 3.10 (m, 1H), 3 48 (dt, J = 4.2, 10.5 Hz, 1H), 5.02 (d, J = 0.9 Hz, 1H), 6 21 (m, 2H); GC-MS (EI, m/z, rel. int. (%)) 319 (M^++1, 1 6), 253 (1.8), 181 (100), 163 (17.4), 115 (20 3), 91 (10.3), 66 (41 3), Analysis calcd for $C_{20}H_{30}O_3$ C, 75.43; H, 9.49. Found. C, 75.55, H, 9.11).

The mother liquor (9.73 g) was dissolved in 80% TFA (30 mL) and stirred for 18 h at room temperature. After evaporation of the solvent under reduced pressure the crude product, containing hydroxy tricyclo lactone **8** (enantiopurity ≈ 69%) was purified by chromatography (SiO_2, hexane / ethyl acetate 9·1) to remove the apolar by-products *l*-menthol and *l*-menthyl trifluoroacetate The product was then quickly eluted from the column (hexane / ethyl acetate 1·1) to give **8** as a solid (4.00 g, 73%). Without further purification **8** was treated with *d*-menthol

under the same conditions as described for the preparation of **9a**. Yield of **10a** (d.e.> 98%) after crystallization from *n*-hexane 3.29 g, 24% (calculated from starting rac. alcohol **8**). Mp 131-132.5 ^{0}C; $[\alpha]_D$ +148^0 (c 0.38, CH_2Cl_2); Analysis calcd for $C_{20}H_{30}O_3$: C, 75.43; H, 9.49. Found: C, 75.35; H, 9.67. ^{1}H-NMR and mass data were the same as for compound **9a**.

5(R)-Hydroxy-2(S)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**8a**)
Enantiopure *l*-menthyloxy lactone **9a** (3.65 g, 11.5 mmol) was dissolved in 80% (v/v) TFA (50 mL) and stirred for 18h at room temperature. After evaporation of the solvent under reduced pressure the crude product was purified by chromatography (SiO_2, hexane / ethyl acetate 9:1) to remove the apolar by-products *l*-menthol and *l*-menthyl trifluoroacetate. The product was then quickly eluted from the column (hexane / ethyl acetate 1:1) to give **8a** as a solid (1.99 g, 97%), which was sufficiently pure for further reactions. An analytical sample was obtained by recrystallization from hexane/ethyl acetate. Mp 180-182^{0}C; $[\alpha]_D$ +21.7^0 (c 0.42, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$. 100 MHz): δ 1.55 (s, 3H), 1.66 (m, 2H), 2.52 (dd, J = 1.2, 4.1 Hz, 1H), 2.82 (m, 1H), 3.14 (m, 1H), 4.94 (br s, 1H), 5.22 (d, J = 1.2 Hz, 1H), 6.22 (m, 2H); GC-MS (EI, m/z, rel. int. (%)): 181 (M^++1, 2.6), 163 (1.3), 115 (7.8), 91 (40.7), 66 (100); Analysis calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.39; H, 6.48).

5(S)-Hydroxy-2(R)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**8b**)
This compound was prepared from *d*-menthyloxy lactone **10a** (3.12 g, 9.80 mmol) in the same way as described for its enantiomer **8a**. Yield 1.71 g, 97%. Mp 173-175^{0}C; $[\alpha]_D$ -21.8^0 (c 0.40, CH_2Cl_2); Analysis calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.41; H, 6.57. ^{1}H-NMR, and mass data were the same as for compound **8a**.

5(S)-Chloro-2(S)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**13a**) *and its 5(R) epimer* (**14a**)
Enantiopure 5(R)-hydroxy lactone **8a** (1.90 g, 10.6 mmol) was dissolved in $SOCl_2$ (10 mL) in the presence of pyridine (0.92 g, 11.6 mmol) at 0^{0}C. The solution was allowed to warm up to room temperature and stirred for 1 h. Excess $SOCl_2$ was removed by evaporation under reduced pressure. The pyridinium HCl salt was removed by filtration and the filtrate was concentrated to dryness. Purification by flash chromatography (hexane / ethyl acetate 9:1) gave *exo*-5(S)-chloro lactone **13a** (1.59 g, 78%) as a solid and *endo*-5(R)-chloro lactone **14a** (272 mg, 13%), which solified on standing. Analytical samples of **13a** and **14a** were obtained by recrystallization from *n*-hexane.
13a Mp 97-99^{0}C; $[\alpha]_D$ -6.7^0 (c 0.64, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$. 100 MHz): δ 1.64 (s, 3H), 1.69 (m, 2H), 2.90 (m, 1H), 3.00 (dd, J = 1.0, 4.2 Hz, 1H), 3.24 (m, 1H), 5.70 (d, J = 1.0 Hz, 1H), 6.23 (m, 2H); GC-MS (EI, m/z, rel. int. (%)): 201/199 (M^++1, 2.3), 163 (7.5), 97 (6.0), 91 (15.2), 66 (100); Analysis calcd for $C_{10}H_{11}O_2Cl$: C, 60.46; H, 5.58. Found: C, 60.48; H, 5.57.
14a Mp 67-68^{0}C; $[\alpha]_D$ -15.9^0 (c 0.4, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$. 100 MHz): δ 1.54 (s, 3H), 1.71 (m, 2H), 2.86 (m, 1H), 2.97 (dd, J = 3.9, 7.0 Hz, 1H), 3.20 (m, 1H), 6.23 (d, J = 7.0 Hz, 1H), 6.24 (m, 1H), 6.44 (m, 1H); Analysis calcd for $C_{10}H_{11}O_2Cl$: C, 60.46; H, 5.58. Found: C, 60.40; H, 5.64.

5(R)-Chloro-2(R)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**13b**) *and its 5(S) epimer* (**14b**)

These compounds were prepared from enantiopure 5(S)-hydroxy lactone **8b** (1.60 g, 8.89 mmol) in the same way as described for the synthesis of **13a** and **14a**. Yield 1.14 g, 66% of *exo*-**13b** as colorless needles and 125 mg, 11% of *endo*-**14b** as a colourless oil, which crystallized on standing.

13b Mp 99^{0}C; $[\alpha]_D$ +8.0^0 (c 0.4, CH_2Cl_2); Analysis calcd for $C_{10}H_{11}O_2Cl$: C, 60.46; H, 5.58. Found: C, 60.64; H, 5.50. ^{1}H-NMR, and mass data were the same as for compound **13a**.

14b Mp 67-68^{0}C; $[\alpha]_D$ +14.2^0 (c 0.47, CH_2Cl_2); Analysis calcd for $C_{10}H_{11}O_2Cl$: C, 60.46; H, 5.58. Found: C, 60.55; H, 5.66. ^{1}H-NMR, and mass data were the same as for compound **14a**.

2(S)-Methyl-5(R)-(2-oxo-3a(R),6a(R)-dihydro-6H-cyclopenta[b]furan-3-ylidenemeth-oxy)-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**16a**) *and its 3a(S),6a(S) diastereomer* (**16b**)

Potassium *tert*-butoxide (139 mg, 1.24 mmol) was added to a solution of racemic hydroxymethylenolactone **15**[19] (180 mg, 1.18 mmol) in dry DMF (6 mL) with stirring at room temperature under nitrogen. To this solution was gradually added *exo*-5(S)-chloro lactone **13a** (213 mg, 1.07 mmol) in dry DMF (4 mL) at room temperature. After 22 h of stirring the reaction mixture was quenched with acetic acid (0.5 mL). DMF was removed *in vacuo* and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified using flash chromatography (SiO_2, hexane / ethyl acetate 3:1) to afford two diastereomeric products. The fast moving diastereomer **16a** (114 mg, 34%) was obtained as a white solid, and crystallization from hexane/ ethyl acetate afforded analytically pure **16a**. The slow moving diastereomer **16b** (128 mg, 38%) was obtained as a white solid, which gave an analytically pure sample after crystallization from hexane/ ethyl acetate.

16a Mp 180-181.5^{0}C; $[\alpha]_D$ +175^0 (c 0.12, $CHCl_3$); ^{1}H-NMR ($CDCl_3$. 400 MHz): δ 1.58 (s, 3H), 1.73 (m, 2H), 2.69 (dm, ^{2}J = 18.6 Hz, 1H), 2.72 (d, J = 4.2 Hz, 1H), 2.80 (dm, ^{2}J = 18.6 Hz, 1H), 2.90 (m, 1H), 3.23 (m, 1H), 4.07 (m, 1H), 5.11 (dt, J = 2.5, 6.4 Hz, 1H), 5.21 (br s, 1H), 5.64 (m, 1H), 5.75 (m, 1H), 6.21 (dd, J = 2.9, 5.7 Hz, 1H), 6.30 (dd, J = 3.0, 5.7 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H); MS (EI, m/z, rel. int. (%)): 315 (M^++1, 0.06), 249 (0.13), 163 (66.0), 153 (5.3), 97 (100), 91 (4.5), 66 (8.4); Analysis calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.67; H, 5.57.

16b Mp 205-207^{0}C; $[\alpha]_D$ -255^0 (c 0.13, $CHCl_3$); ^{1}H-NMR ($CDCl_3$. 400 MHz): δ 1.59 (s, 3H), 1.73 (m, 2H), 2.67 (dm, ^{2}J = 18.5 Hz, 1H), 2.73 (d, J = 4.2 Hz, 1H), 2.80 (dm, ^{2}J = 18.5 Hz, 1H), 2.90 (m, 1H), 3.22 (m, 1H), 4.08 (m, 1H), 5.11 (dt, J = 2.2, 6.6 Hz, 1H), 5.20 (br s, 1H), 5.60 (m, 1H), 5.73 (m, 1H), 6.21 (dd, J = 2.9, 5.7 Hz, 1H), 6.31 (dd, J = 3.0, 5.7 Hz, 1H), 7.33 (d, J = 2.1 Hz, 1H); MS (EI, m/z, rel. int. (%)): 315 (M^++1, 0.76), 249 (0.29), 163 (78.0), 153 (6.4), 97 (100), 91 (4.9), 66 (8.7); Analysis calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.62; H, 5.68.

2(R)-Methyl-5(S)-(2-oxo-3a(R),6a(R)-dihydro-6H-cyclopenta[b]furan-3-ylidenemeth-oxy)-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**16c**) *and its 3a(S),6a(S) diastereomer* (**16d**)

These compounds were prepared in the same way as described for **16a** and **16b**, starting from *exo*-5(R)-chloro lactone **13b** (260 mg, 1.31 mmol) and racemic hydroxymethylenolactone **15**[19] (200 mg, 1.31 mmol). Yield 155 mg, 38% of slow moving diastereomer **16c** as a white solid and

146 mg, 35% of fast moving diastereomer **16d** as a white solid. Both compounds were recrystallized from hexane/ ethyl acetate to obtain analytically pure samples.
16c Mp 211.5^{0}C; $[\alpha]_D$ +262^0 (c 0.18, $CHCl_3$); Analysis calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.81; H, 5.80. ^{1}H-NMR and mass data were the same as for compound **16b**.
16d Mp 181.5-182^{0}C; $[\alpha]_D$ -173^0 (c 0.18, $CHCl_3$); Analysis calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.71; H, 5.60. ^{1}H-NMR and mass data were the same as for compound **16a**.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene)-3,3a(R),6,6a(R)-tetrahydro-cyclopenta[b]furan-2-one (**4a**)
Fast moving cycloadduct **16a** (66 mg, 0.21 mmol) was dissolved in *o*-dichlorobenzene (25 mL) and heated at 180^{0}C for 15 h. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, hexane / ethyl acetate 2:1) to give the diastereomer **4a** (34 mg, 64%) as a white solid. All analytical data (Mp, $[\alpha]_D$, ^{1}H-NMR, and mass data) were in complete agreement with those reported previously.[19]

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene)-3,3a(S),6,6a(S)-tetrahydro-cyclopenta[b]furan-2-one (**4b**)
Prepared starting from the slow moving cycloadduct **16b** (30 mg, 0.095 mmol) in the same way as described for the synthesis of **4a**. Yield 14 mg, 59% of **4b** as a slightly yellow oil. All analytical data ($[\alpha]_D$, ^{1}H-NMR, and mass data) were in complete agreement with those reported.previously.[19]

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(S)-yloxymethylene)-3,3a(R),6,6a(R)-tetrahydro-cyclopenta[b]furan-2-one (**4c**)
Prepared starting from the slow moving cycloadduct **16c** (65 mg, 0.21 mmol) in the same way as described for the synthesis of **4a**. Yield 34 mg, 66% of **4c** as a slightly yellow oil. All analytical data ($[\alpha]_D$, ^{1}H-NMR, and mass data) were in complete agreement with those reported previously.[19]

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(S)-yloxymethylene)-3,3a(S),6,6a(S)-tetrahydro-cyclopenta[b]furan-2-one (**4d**)
Prepared starting from the fast moving cycloadduct **16d** (60 mg, 0.19 mmol) in the same way as described for the synthesis of **4a**. Yield 24 mg, 51% of **4d** as a white solid. All analytical data (Mp, $[\alpha]_D$, ^{1}H-NMR, and mass data) were in complete agreement with those reported previously.[19]

2.4 References and notes

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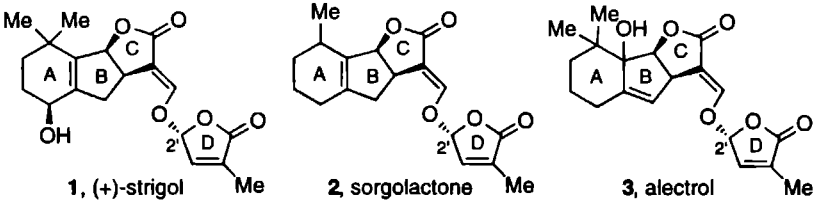
Chapter 3*

Enzymatic Kinetic Resolution of 5-hydroxy-4-oxa *endo*-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-ones: A Useful Approach to D-ring Synthons for Strigol Analogs with Remarkable Stereoselectivity

Abstract: Racemic 5-hydroxy-4-oxa *endo*-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one and its 2-methyl analog were resolved employing a lipase catalyzed acetylation reaction. The latter compound thus gave access to a homochiral D-ring synthon for strigolactones. The enzymatic acetylation reaction occurred with a remarkable inversion of configuration at C-5, through which it is possible to achieve a highly efficient asymmetric synthesis of 5-acetoxy-2(5H)-furanone.

3.2 Introduction

(+)-Strigol **1** and some structurally related sesquiterpene lactones sorgolactone **2** and alectrol **3** are members of the "strigolactone" family,[1] which induce germination of seeds of the parasitic weeds *Striga* and *Orobanche*.[2,3,4]



As part of our interest in the (asymmetric) synthesis of the strigolactones and their synthetic analogs,[5-9] an asymmetric synthesis was devised recently for the tricyclic *exo*-chlorolactone **4a** (scheme 1),[10] which can be regarded as a homochiral latent D-ring synthon. Its preparation as well as its use as a D-ring precursor has been discussed in the preceding chapter. The D-ring is a common structural feature of the strigolactones and is of prime importance for full biological activity. The absolute stereochemistry at C-2' is essential for optimal stimulation of germination.[9,11,12]

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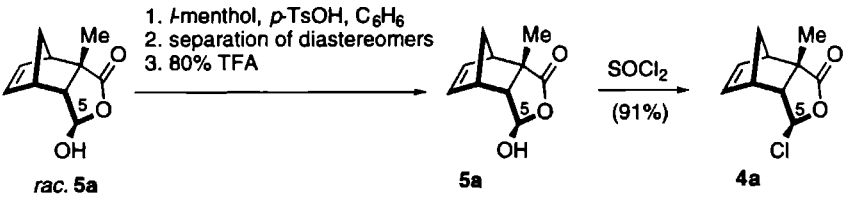
Scheme 1



rac. **5a** **5a** **4a**

The key steps in the synthesis of **4a** involve menthylation with *l*-menthol to give a 1:1 mixture of diastereomeric menthyl ethers, separation of the diastereomers, followed by acidic hydrolysis to give the enantiopure hydroxy lactone **5a**. This method provides access to both enantiomers of **5a** by choosing the appropriate enantiomer of menthol. However, the resolution is quite laborious since it requires two steps and a careful selective recrystallization. Moreover, one equivalent of the chiral auxiliary is required. In order to circumvent these problems, a study was undertaken to improve the resolution, using an enzymatic approach.

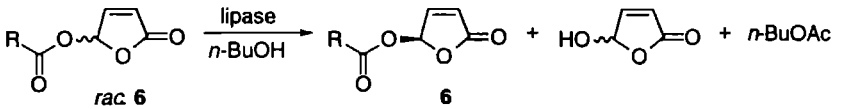
Enzymes currently find widespread use in synthetic organic chemistry.[13a-d] A prominent example of an enzymatic asymmetric transformation is the kinetic resolution of a racemic alcohol R*OH in the presence of an acyl donor $R^2C(O)OR^3$, catalyzed by a lipase. The charm of this methodology lies in the facts that organic solvents can be used, work-up is extremely simple, and a large variety of substrates is tolerated in this transformation. The application of enol esters as irreversible acyl donors[13b] makes this type of resolution even more attractive. This chapter deals with the kinetic resolution of racemic *endo*-tricyclic hydroxy lactones **5** employing vinyl acetate as irreversible acyl donor, catalyzed by lipase PS.

3.2 Results and Discussion

Starting *endo* -tricyclic hydroxy lactones **5** were obtained by standard literature procedures. Hydroxy lactone **5a** was prepared by a Diels-Alder reaction of citraconic anhydride and cyclopentadiene, followed by partial reduction according to the procedure of Canonne (chapter 2).[14] Hydroxy lactone **5b** was obtained by photooxidation of furfural[15] and subsequent Diels-Alder reaction with cyclopentadiene.

Kinetic resolution. In a recent paper Kellogg *et al.* described the lipase mediated transesterification of 5-acyloxy-2(5H)-furanones *rac.* **6** with *n*-butanol resulting in e.e.'s ranging from 68-98% (scheme 2) with hitherto unknown stereochemistry.[16]

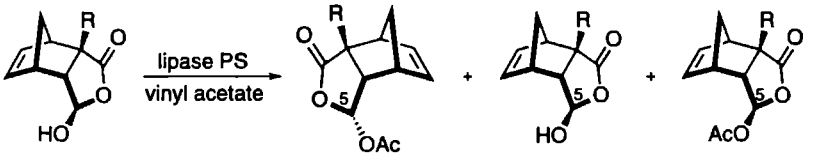
Scheme 2



rac **6** **6**

This study deals with the irreversible acetylation of *endo*-tricyclic hydroxy lactones **5** in the presence of vinyl acetate in dichloromethane, catalyzed by lipase PS (scheme 3). The results are collected in Tables 1 and 2.

Scheme 3



rac. **5a** (R=Me) *rac.* **5b** (R=H) **7a** (R=Me) **7b** (R=H) **5a** (R=Me) **5b** (R=H) **8a** (R=Me) **8b** (R=H)

Table 1. Lipase PS catalyzed transesterification of *endo*-tricyclic hydroxy lactone *rac.* **5a**

entry	time	conversion (%)	product distribution (%)		
			7a (% e.e.)	**5a** (% e.e.)	**8a**
1	22 h.	30.8	30.2 (>90)	69.2 (41)	0.6
2	46 h.	48.0	45.7 (87)	52.0 (79)	2.3
3	70 h.	56.2	51.6 (87)	43.8 (85)	4.6

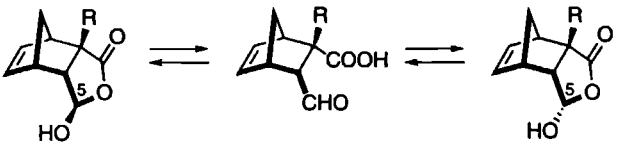
Table 2. Lipase PS catalyzed transesterification of *endo*-tricyclic hydroxy lactone *rac.* **5b**

entry	time	conversion (%)	product distribution (%)		
			7b (% e.e.)	**5b** (% e.e.)	**8b**
1	17 h.	39.0	39.0 (>90)	61.0 (56)	0
2	47 h.	53.5	50.0 (>90)	46.5 (>90)	3.5
3	17 days	60.8	45.2 (>90)	39.2 (>90)	15.6

As can be deduced from the data shown in Tables 1 and 2, the lipase PS mediated acetylation of hydroxy lactones **5** is accomplished in good to excellent e.e.'s. It should be emphasized that this conversion does not take place when other lipases were employed (lipase A, lipase R). Along with the *endo*-acetates **7a** and **7b**, *exo*-acetates **8a** and **8b** were formed in minor amounts (Tables 1 and 2). A striking observation is the fact that this reaction takes place with epimerization at C-5. The formation of the *endo*-acetates **7a** and **7b** could readily be deduced from ^{1}H-NMR analysis. The acetal proton H_5 of the *endo*-isomers **7a** and **7b** exhibited a doublet (^3J = 7 Hz for **7a** and 6 Hz for **7b**) at ca. 0.6 ppm lower field as compared to the corresponding *exo*-isomers (^3J = 1 Hz), which is in agreement with previous observations.[10] These results suggest that the reaction takes place via the thermodynamically unfavorable *endo*-hydroxy epimers **9**, which can be formed from the corresponding *exo* isomers by **mutarotation** (scheme

4). During NMR experiments in $CDCl_3$, the presence of the *endo*-epimers in the solution could not be observed.

Scheme 4



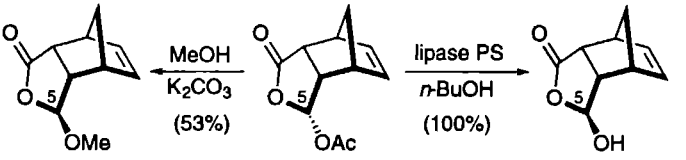
5a (R=Me)
5b (R=H)

9a (R=Me)
9b (R=H)

It should be noted that it is not possible to obtain the *endo*-acetates by any other means. Acetylation reactions under conventional conditions, such as Ac_2O/pyridine or Ac_2O/*p*-TsOH gave exclusively the *exo*-acetates **8**. In order to gain information about the existence of the *exo*/*endo* equilibrium (scheme 4), the *endo*-acetate **7b** was subjected to a transesterification reaction. However, employing MeOH as a solvent in the presence of K_2CO_3 the expected *exo*-hydroxy lactone *ent.* **5b** was not obtained, but *exo*-methoxy lactone *ent.* **10b** was isolated as the main product (scheme 5). Apparently, the initially formed hydroxy lactone *ent.* **5b** undergoes an acetalization under the reaction conditions. Therefore, the enzyme mediated method was investigated. Lipase PS catalyzed transesterification in the presence of 10 eq. *n*-BuOH in CH_2Cl_2 led to the exclusive formation of *exo*-hydroxy lactone *ent.* **5b** (scheme 5). Again, no trace of *endo*-hydroxy lactone could be detected.

A convenient method for the conversion of acetate **7a** into the hydroxy lactone *ent.* **5a** is treatment with 80% TFA (yield 84%). Lactone *ent.* **5a** is the precursor of chloro lactone *ent.* **4a** (scheme 1) as has been described in detail in chapter 2. It is remarkable that these reaction conditions do not work for the conversion of **7b** into *ent.* **5b**.

Scheme 5



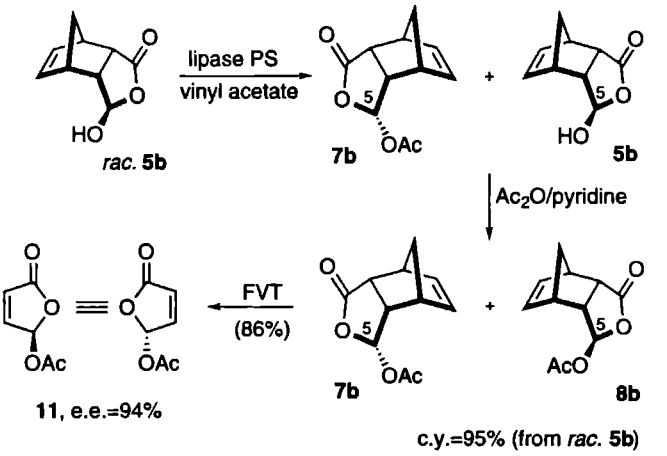
ent. **10b** **7b** *ent.* **5b**

The results obtained with lipase PS catalyzed acetylation of racemic hydroxy lactones **5** (scheme 3) fit into a model in which only one enantiomer of the thermodynamically unfavorable *endo* hydroxy lactones **9** is withdrawn from the *exo*/*endo* equilibrium (scheme 4) to undergo a relatively fast enzymatic acetylation reaction. This sequence is an example of the **Curtin-Hammett principle**. This remarkably large kinetic difference between the *endo*- and *exo*-

hydroxy lactones results in an excellent selectivity of product formation. It should be noted that in the absence of the lipase no conversion into **7a,b** or **8a,b** was observed even after 17 days. This implies that the formation of *exo*-acetates **8a,b** (*e.g.* Table 2, entry 3) is also catalyzed by the lipase, albeit at a much lower rate. The formation of the *exo*-acetates **8a** and **8b**, which are diastereomeric to the initially formed products **7a,b**, takes place via the *exo*-epimers **5a** and **5b**, respectively. This formation of diastereomers **7** and **8**, which is the ultimate result of the *exo/endo* equilibrium as depicted in scheme 4, is quite unusual in kinetic resolutions.

The interesting finding shown in scheme 3 can be advantageously utilised to achieve a sequence with full chiral economy (scheme 6) in the following manner.

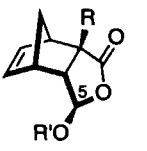
Scheme 6



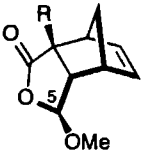
The crude mixture of **7b** and **5b**, obtained by kinetic resolution of *rac.* **5b** is acetylated under standard conditions to give the diastereomeric products **7b** and **8b**. Without further purification this mixture was subjected to a cycloreversion reaction, employing the technique of flash vacuum pyrolysis (FVT). This reaction led to the formation of one single isomer of 5-acetoxy-2(5H)-furanone **11**. This remarkable result can be rationalized by taking into account that a double stereodifferentiation has taken place. These results demonstrate the successful application of an enzymatic kinetic resolution of a racemic mixture, providing one single enantiomer without purification of any intermediate.

Determination of e.e. and absolute configuration. The e.e.'s of the tricyclic hydroxy lactones **5a** and **5b** were established after menthylation with *l*-menthol to give the corresponding *l*-menthyloxy lactones **12a** and **12b** as a mixture of diastereomers with known absolute stereochemistry.[10,17] The d.e.'s could thus be determined by comparison of the relative intensities of the acetal H_5 proton signals in the ^{1}H-NMR spectrum. As there is no stereochemical preference in the menthylation reaction,[10] this derivatization allows the determination of the e.e.'s of the hydroxy lactones **5**. Moreover, this derivatization to menthyl acetals **12** with known

stereochemistry enables the unambigious assignment of the absolute stereochemistry as is shown (scheme 3). Although effective, a more convenient procedure to determine the respective e.e.'s involves the conversion of hydroxy lactones **5** and *endo*-acetoxy lactones **7** into the corresponding methyl acetals **10a,b** and *ent.* **10a,b**. These methylations occurred with complete *exo*-selectivity in almost quantitative yields.



10a (R=Me, R'=Me)
10b (R=H, R'=Me)
12a (R=Me, R'=Menth(*l*)
12b (R=H), R'=Menth(*l*)



ent. **10a** (R=Me)
ent. **10b** (R=H)

The e.e.'s then were determined employing 400 MHz ^{1}H-NMR analysis in the presence of the chiral shift reagent Eu(hfc)$_3$ (1.5 eq.). In the case of methoxy lactones **10a** and *ent.* **10a** a difference of 0.03 ppm was observed for the α-methyl protons. On the other hand, the e.e. of methoxy lactone **10b**[18] was calculated on the basis of a 0.03 ppm difference of chemical shift of the acetal proton H_5 as compared to its enantiomer *ent.* **10b**. The determination of e.e. of acetoxy-2(5H)-furanone **11** was accomplished by comparison of the relative intensities of the CH_3 signals in the ^{1}H-NMR spectrum using 0.4 eq. of Eu(hfc)$_3$, which resulted in a downfield shift of approximately 0.8 ppm and a difference of 0.16 ppm for both enantiomers. On the basis of the above assignment of the absolute stereochemistry the levorotatory 5-acetoxy-2(5H)-furanone **11**, obtained by Kellogg *et al.* according to scheme 2,[16] can be assigned as 5(R).

3.3 Conclusion

Lipase PS mediated acetylation proved to be a simple, highly efficient method for the kinetic resolution of racemic tricyclic hydroxy lactones **5**. Employing this methodology it is possible to synthesize both enantiomers of *exo*-chlorolactones **4a**. These optically active latent butenolides are useful synthons for the preparation of homochiral strigolactones.[10] The kinetic resolution was accompanied with a remarkable epimerization, which could be used to demonstrate the synthesis of enantiopure 5-acetoxy-2(5H)-furanone **11** with optimal "chiral economy".

3.4 Experimental Section

General remarks

For general methods and instrumentation, see ref. 10. GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30m x 0.25 mm). Helium was used as carrier gas, and electron impact (EI) was used as ionization mode. Lipase PS was obtained from Amano as a gift.

General procedure for the enzymatic kinetic resolution of the tricyclic hydroxy lactones *rac.* 5a and *rac.* 5b.

To a solution containing *exo*-hydroxy tricyclic lactone *rac.* **5a**[14] (500 mg, 2.79 mmol) and vinyl acetate (2.57 mL, 27.9 mmol) in CH_2Cl_2 (25 mL) was added lipase PS (1.0 g) and powdered 4Å molecular sieves (0.5 g). The suspension was stirred vigorously at room temperature. At given intervals (Tables 1 and 2) samples were taken (3 mL) and filtered over hyflo. The hyflo was washed with CH_2Cl_2 and the crude mixture was analyzed by 100 MHz ^{1}H-NMR ($CDCl_3$) for conversion. Purification by chromatography (SiO_2, hexane/ethyl acetate 3:1) afforded *endo*-acetate **7a** as a white solid and *exo*-alcohol **5a** as a white solid, which were analyzed for e.e. (*vide infra*).

Enantiomeric excess determination.

The hydroxy lactones **5a** and **5b** were transformed into the corresponding *l*-menthyl ethers **12**.[10,17] Alternatively, **5a** and **5b** were converted into the corresponding *exo* methoxy lactones **10a**, **10b** and subsequently analyzed by 400 MHz ^{1}H-NMR ($CDCl_3$) in the presence of ca. 1.5 eq. $Eu(hfc)_3$ (*vide infra*). Similarly, *endo*-acetates **7a** and **7b** were methylated to give *ent.* **10a** and *ent.* **10b** respectively (*vide infra*), which were analyzed for e.e. in the same manner.

5(R)-Acetoxy-2(R)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**7a**) *and 5(R)-hydroxy-2(S)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one* (**5a**)

These compounds were synthesized according to the general procedure starting from *rac.* **5a**[14] (3.00 g, 16.7 mmol). The reaction was stopped after 73h. Purification by chromatography (SiO_2, hexane/ethyl acetate 3:1) gave **7a** (1.28 g, 34%) as a white solid and **5a** (1.18 g, 39%) as a white solid. Analytical samples of **5a** and **7a** were obtained by recrystallization from hexane/ethyl acetate.

7a Mp 98.5-101.5^{0}C; $[\alpha]_D$ -88.4^0 (c 0.4, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.54 (s, 3H), 1.69 (m, 2H), 2.15 (s, 3H), 2.85 (m, 1H), 2.87 (dd, J = 3.9, 7.0 Hz, 1H), 3.04 (m, 1H), 6.26 (m, 2H), 6.50 (d, J = 7.0 Hz, 1H); GC-MS (EI, m/z, rel. int. (%)): 163 (M^+-OAc, 90.4), 157 (1.7), 152 (23.4), 97 (13.6), 91 (16.9), 66 (100); Analysis calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 65.28; H, 6.31.

5a All analytical data (Mp, $[\alpha]_D$, ^{1}H-NMR, and mass data) were in complete agreement with those reported previously.[10]

5(R)-Acetoxy-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (**7b**) *and 5(R)-Hydroxy-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one* (**5b**)

These compounds were synthesized according to the general procedure starting from *rac.* **5b**[14] (3.00 g, 18.1 mmol). The reaction was stopped after 46h. Purification by chromatography (SiO_2, hexane/ethyl acetate 3:1) gave **7b** (1.65 g, 44%) as a white solid and **5b** (1.41 g, 47%) as a white

solid. Analytical samples of **5b** and **7b** were obtained by recrystallization from hexane/ethyl acetate.
7b Mp 116.5-118^{0}C; $[\alpha]_D$ -126.0^0 (c 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.47 (dt, J = 1.0 Hz, 9.0 Hz, 1H), 1.65 (dt, J = 1.0 Hz, 9.0 Hz, 1H), 2.15 (s, 3H), 3.11 (m, 1H), 3.36 (m, 3H), 6.25 (m, 2H), 6.48 (d, J = 6.0 Hz, 1H); GC-MS (EI, m/z, rel. int. (%)): 166 (M^++1-Ac, 12.2), 149 (M^+-OAc, 49.2), 137 (12.2), 91 (42.3), 83 (9.1), 66 (100); Analysis calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.55; H, 5.79.
5b Mp 134-136.5^{0}C; $[\alpha]_D$ +53.2^0 (c 0.2, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.37 (dt, J = 1.0 Hz, 8.5 Hz, 1H), 1.56 (dt, J = 1.0 Hz, 8.5 Hz, 1H), 2.86 (m, 1H), 3.33 (m, 3H), 4.83 (br s, 1H), 5.16 (br s, 1H), 6.14 (m, 2H); GC-MS (EI, m/z, rel. int. (%)): 167 (M^++1, 1.9), 149 (2.0), 91 (29.3), 83 (3.1), 66 (100); Analysis calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 64.97; H, 6.00.

5(S)-hydroxy-2(R)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (*ent.* **5a**)
Enantiopure *endo*-acetoxy lactone **7a** (251 mg, 1.13 mmol) was dissolved in 80% (v/v) TFA (2 mL) and stirred for 24h at 40^{0}C. After evaporation of the solvent under reduced pressure the crude product was purified by chromatography (SiO_2, hexane/ethyl acetate 3:1) to give *ent.* **5a** as a solid (268 mg, 84%), which was sufficiently pure for further reactions. An analytical sample was obtained by recrystallization from hexane/ethyl acetate. Mp 180-182^{0}C; $[\alpha]_D$ +21.7^0 (c 0.42, CH_2Cl_2); ^{1}H-NMR and mass data were the same as for compound **5a**.

5(S)-Hydroxy-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (*ent.* **5b**)
A solution containing **7b** (50 mg, 0.24 mmol) and *n*-BuOH (0.22 mL, 24 mmol) in CH_2Cl_2 (3 mL) was treated with lipase PS (100 mg) and powdered 4Å molecular sieves (50 mg). The suspension was stirred vigorously at room temperature. After 24 h the suspension was filtered over hyflo, washed with CH_2Cl_2 and the filtrate was concentrated *in vacuo*. Yield 39.0 mg, 98% of pure *ent.* **5b** as a white solid. An analytical sample was obtained by recrystallization from hexane/ethyl acetate. Mp 130.5-131.5^{0}C; $[\alpha]_D$ -48.6^0 (c 0.2, CH_2Cl_2); ^{1}H-NMR and mass data were the same as for compound **5b**.

Racemic exo-5-methoxy-2-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (*rac.* **10a**)
For the determination of the e.e. of 5(R)-hydroxy lactone **5a**. *Rac.* **5a** (50 mg, 0.28 mmol) was treated with methanol (2 mL) and 1 drop of thionyl chloride. The solution was stirred for 30 min. and concentrated *in vacuo* to give pure *rac.* **10a** (53.4 mg, 96%) as a white solid. Mp 86.5-89.5^{0}C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.45 (s, 3H), 1.59 (m, 2H), 2.40 (dd, J = 1.0, 4.2 Hz, 1H), 2.75 (m, 1H), 3.05 (m, 1H), 3.36 (s, 3H), 4.66 (d, J = 1.0 Hz, 1H), 6.10 (m, 1H), 6.19 (m, 1H); Addition of 1.5 eq. of the chiral shift reagent Eu(hfc)$_3$ gave a splitting of the α-methyl signal of 0.03 ppm (1.07 ppm downfield shift). GC-MS (EI, m/z, rel. int. (%)): 195 (M^++1, 59.0), 163 (10.4), 135 (15.2), 129 (39.4), 97 (20.1), 91 (26.9), 66 (100); Analysis calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.26. Found: C, 67.80; H, 7.19.

5(S)-Methoxy-2(R)-methyl-4-oxa-endo-tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (*ent.* **10a**)
For the determination of the e.e. of *endo*-5(R)-acetoxy lactone **7a.** A solution of **7a** (25 mg, 0.11 mmol) in methanol (2 mL) was treated with 1 drop of thionyl chloride. The solution was stirred for 30 min. and concentrated *in vacuo* to give pure *ent.* **10a** (21.1 mg, 97%), which was analyzed for e.e. as described for *rac.* **10a.**

Racemic exo-5-methoxy-4-oxa-endo-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (*rac.* **10b**)[18]
For the determination of the e.e. of 5(R)-hydroxy lactone **5b**. A solution of *rac.* **5b** (50 mg, 0.31 mmol) in methanol (2 mL) was treated with 1 drop of thionyl chloride. The solution was stirred for 30 min. and concentrated *in vacuo* to give crude *rac.* **10b**, which was not sufficiently pure for e.e. determination. Purification by chromatography (SiO_2, hexane/ethyl acetate 9:1) gave pure *rac.* **10b** (47.2 mg, 84%) as a white solid. Mp 54.5-55.5^{0}C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.44 (dt, J = 1.0 Hz, 8.6 Hz, 1H), 1.62 (dt, J = 1.0 Hz, 8.6 Hz, 1H), 2.91 (m, 1H), 3.19 (m, 1H), 3.31 (m, 2H), 3.43 (s, 3H), 4.79 (d, J = 1.1 Hz, 1H), 6.20 (m, 1H), 6.25 (m, 1H); Addition of 1.5 eq. of the chiral shift reagent Eu(hfc)$_3$ gave a splitting of the signal of the acetal proton H_5 of 0.03 ppm (1.37 ppm downfield shift). GC-MS (EI, m/z, rel. int. (%)): 181 (M^++1, 10.7), 149 (12.6), 121 (14.4), 115 (9.4), 91 (54.6), 83 (15.3), 66 (100); Analysis calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.12; H, 6.62.

5(S)-Methoxy-4-oxa-endo-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (*ent.* **10b**)
For the determination of the e.e. of *endo*-5(R)-acetoxy lactone **7b.** This compound was prepared from **7b** (40 mg, 0.19 mmol) in the same way as described for the synthesis of *ent.* **10a**. Yield after chromatography (SiO_2, hexane/ethyl acetate 9:1) 28.2 mg, 83 %. The e.e. was determined according to the procedure as described for *rac.* **10b**.

Racemic exo-5-acetoxy-2-methyl-4-oxa-endo-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (*rac.* **8a**)
Rac. **5a** (100 mg, 0.56 mmol) was dissolved in pyridine/acetic anhydride 2:1 v/v (1 mL) and stirred for 17 h at room temperature. The solvents were removed *in vacuo* and the residue was coevaporated with toluene. Yield 121.8 mg, 98% of pure *rac.* **8a** as a colorless oil. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.50 (s, 3H), 1.62 (m, 2H), 2.04 (s, 3H), 2.50 (dd, J = 0.9, 4.2 Hz, 1H), 2.80 (m, 1H), 3.12 (m, 1H), 5.87 (d, J = 0.9 Hz, 1H), 6.19 (m, 2H), GC-MS (EI, m/z, rel. int. (%)): 163 (M^+-OAc, 36.3), 157 (3.0), 97 (18.2), 91 (11.5), 66 (100); HRMS/EI: m/z calcd for $C_{12}H_{14}O_4$: 222.0892. Found 222.08931 ± 0.00088.

Racemic exo-5-acetoxy-4-oxa-endo-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (*rac.* **8b**)
This compound was prepared from *rac.* **5b** (100 mg, 0.60 mmol) in the same way as described for the synthesis of *rac.* **8a**. Purification by chromatography (SiO_2, hexane/ethyl acetate 3:1) afforded *rac.* **8b** (119.8 mg, 87%) as a white solid. An analytically pure sample was obtained by recrystallization from hexane/ethyl acetate. Mp 82.5-84^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.39 (dt, J = 1.0 Hz, 8.7 Hz, 1H), 1.60 (dt, J = 1.0 Hz, 8.7 Hz, 1H), 2.03 (s, 3H), 2.96 (m, 1H), 3.25 (m, 3H), 5.86 (d, J = 1.2 Hz, 1H), 6.19 (m, 2H); GC-MS (EI, m/z, rel. int. (%)): 149 (M^+-OAc, 10.2), 143 (1.6), 91 (23.6), 83 (11.6), 66 (100); Analysis calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.50; H, 5.79.

5(R)-Acetoxy-2(S)-methyl-4-oxa-endo-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (**8a**)
This compound was prepared from **5a** (100 mg, 0.56 mmol) in the same way as described for the synthesis of *rac.* **8a**. Yield 123.1 mg, 99% of **8a** as a colorless oil. $[\alpha]_D$ -79.3^0 (c 0.4, CH_2Cl_2). ^{1}H-NMR and mass data were the same as for compound *rac.* **8a**.

5(R)-Acetoxy-4-oxa-endo-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (**8b**)
This compound was prepared from **5b** (100 mg, 0.60 mmol) in the same way as described for the synthesis of *rac.* **8b**. Yield 119.8 mg, 87% of **8b** as a white solid. An analytically pure sample

was obtained by recrystallization from hexane/ethyl acetate. Mp 84-86.5^{0}C; $[\alpha]_D$ -27.2^0 (c 0.4, CH_2Cl_2). ^{1}H-NMR and mass data were the same as for compound *rac.* **8b**.

5(R)-Acetoxy-2(5H)-furanone (**11**)
Flash vacuum thermolysis of **7b** (52.4 mg, 0.25 mmol) [sample temp: 80^{0}C; oven temp: 500^{0}C; cold trap temp: -78^{0}C; pressure: 5*10^{-2} mbar] provided pure **11** (32.7 mg, 92%) as a colorless oil. $[\alpha]_D$ -30.9^0 (c 0.7, CH_2Cl_2); ^{1}H-NMR data were the same as reported for *rac.* **11**[19]; Addition of $Eu(hfc)_3$ (0.4 eq) gave a separation of CH_3 signals amounting 0.16 ppm for the corresponding enantiomers (0.8 ppm downfield shift), e.e. 94%.
The same compound **11** was obtained by FVT [sample temp: 120^{0}C; oven temp: 500^{0}C; cold trap temp: -78^{0}C; pressure: 5*10^{-2} mbar] starting from a 1:1 mixture of diastereomeric acetates **7b** and **8b** (110 mg, 0.53 mmol). Yield 64.9 mg, 86% as a colorless oil. $[\alpha]_D$ -34.2^0 (c 0.5, CH_2Cl_2), e.e. 94%

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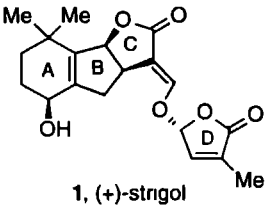
Chapter 4*

Lipase Catalyzed Dynamic Kinetic Resolution of some 5-Hydroxy-2(5H)-Furanones

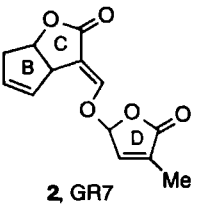
Abstract: Lipase PS was successfully applied in the dynamic kinetic resolution of 5-hydroxy-2(5H)-furanones **4a-d** (quantitative conversions, e e 's 78-86%)

4.1 Introduction

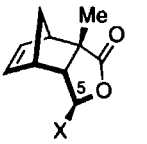
The naturally occurring strigolactones, *e.g* **1**, and their synthetic analogs, *e g* **2**, are highly potent germination stimulants of seeds of several parasitic weeds, such as *Striga* and *Orobanche* spp [1-3] In connection with our interest in the stereocontrolled synthesis of these germination stimulants enantiopure D-ring precursors are required Recently, the preparation of all four stereoisomers of GR7 was reported[4] using the latent D-ring **3b**, which was resolved in its enantiomers via the menthyloxy derivative **3a**, as is outlined in chapter 2 This procedure was improved by conducting a lipase catalyzed acetylation of **3c**, resulting in a highly efficient resolution with concomitant epimerization of the labile stereogenic center C-5 (chapter 3)



1, (+)-strigol



2, GR7



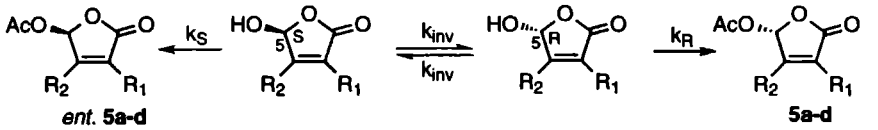
3a, X = O-Menthyl
3b, X = Cl
3c, X = OH

This chapter deals with a similar enzymatic approach for the preparation of potential D-ring precursors of high enantiopurity by the kinetic resolution of 5-hydroxy-2(5H)-furanones **4a-d** as is depicted in scheme 1

*Part of this chapter has been published in Thuring, J W J F , Klunder, A J H , Nefkens, G H L , Wegman, M A , Zwanenburg, B *Tetrahedron Lett* , **1996**, *37*, 4759 4760

4.2 Results and discussion

Scheme 1



4a, $R_1=R_2=H$; **b**, $R_1=R_2=Me$; **c**, $R_1=Me$, $R_2=H$; **d**, $R_1=H$, $R_2=Me$

lipase PS; $H_2C=CHOAc$ (10 equiv.) in CH_2Cl_2

In this lipase mediated dynamic kinetic resolution vinyl acetate was used as the irreversible acyl donor (scheme 1).[5] As a consequence of the labile stereogenic center at C-5, it was expected that a single enantiomer of **5** can be obtained in a theoretical yield of 100%. The interconversion of both enantiomers of **4** by mutarotation allows a dynamic kinetic resolution.[6] The highly rewarding results are collated in the Table. The retro-reaction, *viz* transesterification of *rac.* **5a** in the presence of a lipase to give *rac.* **4a** and either enantiopure **5a** or *ent.* **5a**, has recently been reported.[7] However, this conventional kinetic resolution suffers from an inherent drawback that the maximum yield of a single enantiomer is only 50%.

Table. Dynamic Kinetic Resolution of 5-Hydroxy-2(5H)-Furanones **4a-d**

entry	substrate	reaction time[a]	temp. (^{0}C)	e.e. (%)[b]	enantiomer[c]
1	**4a**	100 h	32	83	(-) **5a**
2	**4a**	150 h	20	84	(-) **5a**
3	**4b**	28 d	35	78	(-) **5b**
4	**4c**	166 h	30	84	(-) **5c**
5	**4d**	168 h	30	86	(-) **5d**

a) reaction time after 100% conversion as determined by capillary G.C. b) determined by chiral G.C., using a β-DEX 120 Capillary Column. c) sign of $[\alpha]_D$ of major enantiomers **5a-d** in dichloromethane.

The data in the Table reveal that the asymmetric transformation of **4a-d** takes place with 100% conversion and with high enantioselectivity. Methyl substituents (entries 3-5) have a negative effect on the reaction rate. Higher temperatures enhance the reaction rate considerably, without affecting the e.e.'s (entries 1-2). Other solvents such as THF or CH_3CN instead of CH_2Cl_2 result in slower reactions and slightly lower e.e.'s (data not shown). It is important to note that the e.e.'s remain constant throughout the reactions as was established by analyzing aliquots at several time intervals. This implies that the stereoinversion (k_{inv}) is infinitely faster than the acetylation reaction ($k_{inv} >> k_R$ and k_S; scheme 1).

Determination of absolute configuration

The absolute stereochemistry of (-)**5a** could readily be deduced by comparison with an authentic sample of known configuration.[8] The absolute configuration of (-)**5b-d** was established by correlation using their chiroptical properties. Recently, Gawronski *et al.* reported the CD data of *ent.* **5a** and a series of chiral 5-substituted 2(5H)-furanones.[9] It was concluded that the absolute configuration at C-5 may be deduced directly from the observed Cotton effects resulting from the n-π^* (235-250 nm) and π-π^* (200-215 nm) transitions of the α,β-unsaturated lactone chromophore. Hence, in the case of 5-acetoxy 2(5H)-furanones **5a-d**, a negative CD for the n-π^* transition and a positive CD for the π-π^* transition correspond to the 5(R) configuration. The CD spectra of **5b-d**, which are depicted in fig. 1, are in full accordance with that of *ent.* **5a**[9] (opposite sign), thus indicating the absolute stereochemistry as is shown (scheme 1).

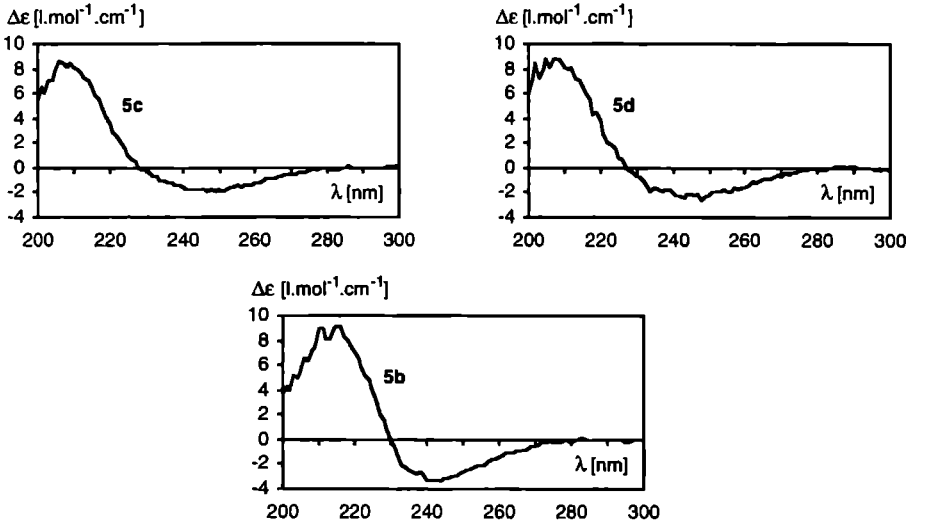


Figure 1. CD-spectra of **5b** (c 0.074 mM, e.e. 78%), **5c** (c 0.13 mM, e.e.>95%), **5d** (c 0.058 mM, e.e. 86%), recorded in acetonitrile

In conclusion, the dynamic kinetic resolution of **4a-d** offers an attractive route to the enantioselective synthesis of a series of 5-acetoxy-2(5H)-furanones **5a-d** with optimal chiral economy. The compounds **5a-d** can be used for the introduction of various D-rings into strigolactones and their analogs in an enantiocontrolled manner. In addition these compounds are chiral synthons with rich prospects.

4.3 Experimental

General remarks
100 MHz ^{1}H-NMR and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 and Bruker AM-400 spectrometer, respectively (Me_4Si as internal standard). All coupling constants are given as ^3J in Hz, unless indicated otherwise. For mass spectra a double focussing VG7070E mass spectrometer was used. GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30m x 0.25 mm). Helium was used as carrier gas, and electron impact (EI) was used as ionization mode. GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas. For the determination of enantiopurities of **5a, 5c,d** a chiral β-DEX 120 capillary GC column (from Suppelco) was used. Nitrogen was the carrier gas (0.7 ml/min, 0.8 atm.) and the oven temperature was 150^{0}C for **5a,c** and 140^{0}C for **5d**. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. CD-spectra were recorded using a Jasco J600 spectrophotometer. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.
Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Hexane was distilled from CaH_2. Ethyl acetate was distilled from potassium carbonate. 5-Hydroxy-2(5H)-furanones **4a**[10], **4b**[11], and **4d**[12] were prepared following established procedures. Compound **4c** was prepared by photo-oxidation ($^1O_2$, MeOH)[13] of 3-methyl-2-furoic acid.[14]

General procedure for the lipase PS catalyzed acetylation of hydroxy-2(5H)-furanones 4a-d
Lipase PS (200 mg) and powdered molecular sieves (4Å) were added to a stirred solution containing **4a-d** (2.0 mmol), vinyl acetate (10 equiv.) and biphenyl (50 mg) as internal standard, in dichloromethane (10 mL). At several time intervals samples (0.5 mL) were taken, filtered over hyflo and analyzed for conversion and e.e. by GC.

Analytical data of 5-acetoxy-2(5H)-furanones 5a-d:
5(R)-Acetoxy-2(5H)-furanone (**5a**)
$[\alpha]_D$ -33.9 (c 0.42, CH_2Cl_2, e.e. 84%); GC-MS (EI, m/z, rel. int. (%)): 142 ($[M]^+$, 6.4), 83 ($[C_4H_3O_2]^+$, 94.8), 43 ($[C_2H_3O]^+$, 100); ^{1}H-NMR data were in agreement with those reported previously.[9]

5(R)-Acetoxy-3,4-dimethyl-2(5H)-furanone (**5b**)
Mp 39-40.5^{0}C (from hexane/ethyl acetate); $[\alpha]_D$ -68.8 (c 0.35, CH_2Cl_2, e.e. 78%); CD (c 0.074 mM, CH_3CN, e.e. 78%) λ_{max} ($\Delta\varepsilon$): 242 (-3.35), 213 (+9.21); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.87 (br s, 3H, =CC$\underline{H}_3$), 1.97 (br s, 3H, =CC$\underline{H}_3$), 2.17 (s, 3H, OC(O)C$\underline{H}_3$), 6.73 (br s, 1H, OC$\underline{H}$O) ppm; GC-MS (EI, m/z, rel. int. (%)): 170 ($[M]^+$, 8.5), 111 ($[C_6H_7O_2]^+$, 100), 43 ($[C_2H_3O]^+$, 76.0); Analysis calcd for $C_8H_{10}O_4$: C, 56.47; H, 5.92. Found: C, 56.44; H, 5.82. The e.e. was determined by ^{1}H-NMR ($CDCl_3$, 400 MHz) in the presence of chiral shift reagent Eu(hfc)$_3$ (0.1 equiv.), which gave a downfield shift of the C(O)C$\underline{H}_3$ signal, amounting 0.18 and 0.23 ppm for the antipodes.

5(R)-Acetoxy-3-methyl-2(5H)-furanone (**5c**)
$[\alpha]_D$ -33.8 (c 0.36, CH_2Cl_2, e.e. 84%); CD (c 0.13 mM, CH_3CN, e.e.>95%) λ_{max} ($\Delta\varepsilon$): 248 (-1.92), 208 (+8.33); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.99 (m, 3H, =CC$\underline{H}_3$), 2.15 (s, 3H, OC(O)C$\underline{H}_3$), 7.00 (m, 2H, OC$\underline{H}$O + =C$\underline{H}$) ppm; GC-MS (EI, m/z, rel. int. (%)): 156 ([M]$^+$, 3.2), 97 ([$C_5H_5O_2$]$^+$, 100), 43 ([C_2H_3O]$^+$, 52.9); HRMS/EI: m/z calcd for $C_7H_8O_4$ 156.0423. Found: 156.04223±0.00090 amu

5(R)-Acetoxy-4-methyl-2(5H)-furanone (**5d**)
$[\alpha]_D$ -85.3 (c 0.1, CH_2Cl_2, e.e. 86%); CD (c 0.058 mM, CH_3CN, e.e. 86%) λ_{max} ($\Delta\varepsilon$): 246 (-2.57), 207 (+8.76); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.10 (m, 3H, =CC$\underline{H}_3$), 2.19 (s, 3H, OC(O)C$\underline{H}_3$), 5.98 (m, 1H, =C$\underline{H}$), 6.79 (m, 1H, OC$\underline{H}$O) ppm; GC-MS (EI, m/z, rel. int. (%)): 156 ([M]$^+$, 7.2), 97 ([$C_5H_5O_2$]$^+$, 100), 43 ([C_2H_3O]$^+$, 44.0); HRMS/EI: m/z calcd for $C_7H_8O_4$ 156.0423. Found: 156.04222±0.00092 amu

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Chapter 5*

Asymmetric Synthesis of all Stereoisomers of the Strigol Analogue GR24. Dependence of the Absolute Configuration on the Stimulatory Activity of *Striga hermonthica* and *Orobanche crenata* seed germination

Abstract: All four enantiopure stereoisomers of the strigol analogue GR24 were prepared *via* two different routes In the first approach enantiopure ABC-fragments **4** were used as the chiral source, whereas in the alternative route both antipodes of the latent D-ring **6** were employed Bioassays revealed significant differences in activity between the four stereoisomers in the stimulation of germination of the parasitic weeds *Striga hermonthica* and *Orobanche crenata*

5.1 Introduction

The strigolactones[1] comprise a class of structural related compounds that are highly potent inducers of seed germination of several parasitic weed species These compounds are exuded by the roots of their hosts The most prominent member of this family, *viz* (+)-strigol **1** (fig 1), has been isolated from the root exudate of the false host cotton (*Gossypium hirsutum* L) [2] Its structure (relative configuration) was elucidated in 1972[3] and the absolute configuration was unambigiously determined several years later [4] Recently, it has been shown to be the major *Striga* germination stimulant produced by the true hosts maize (*Zea mays* L) and proso millet (*Panicum miliaceum* L) [5] It was demonstrated that the absolute stereochemistry of strigol **1** is of prime importance with respect to its seed germination activity [6,7]

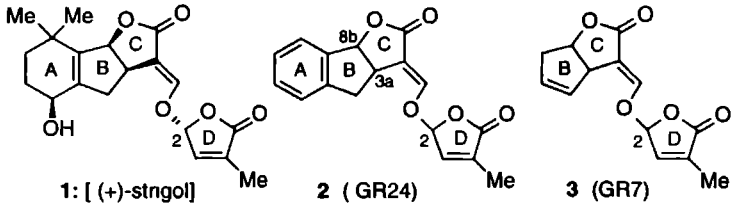


Figure 1

*This chapter has been submitted for publication in *J Agric Food Chem.* by Thuring, J W J F , Nefkens, G H L , Zwanenburg, B

In a study to design simpler analogues of (+)-strigol **1** with optimal bioactivity, Johnson *et al.*[8,9] have prepared the relatively readily accessible synthetic analogue GR24 (**2**) (figure 1). Its racemic preparation and the separation of its diastereomers has been improved considerably.[10,11] The seed germination stimulatory activity toward several parasitic weed species is within the same order of magnitude as that of strigol.[7,12,13] For these reasons GR24 (**2**) has found widespread applications, including its use as a positive control in bioassays of *Orobanche* and *Striga* seed germination.[14] So far, only little attention has been paid to the influence of the absolute stereochemistry of synthetic strigol analogues on the biological activity. Recently, all four stereoisomers of GR7 (**3**), a highly active strigol analogue lacking the A-ring, have been prepared and tested for seed germination activity.[15] It was concluded that the correct absolute stereochemistry at C-2' (R-configuration) is essential to exert maximal bioactivity.

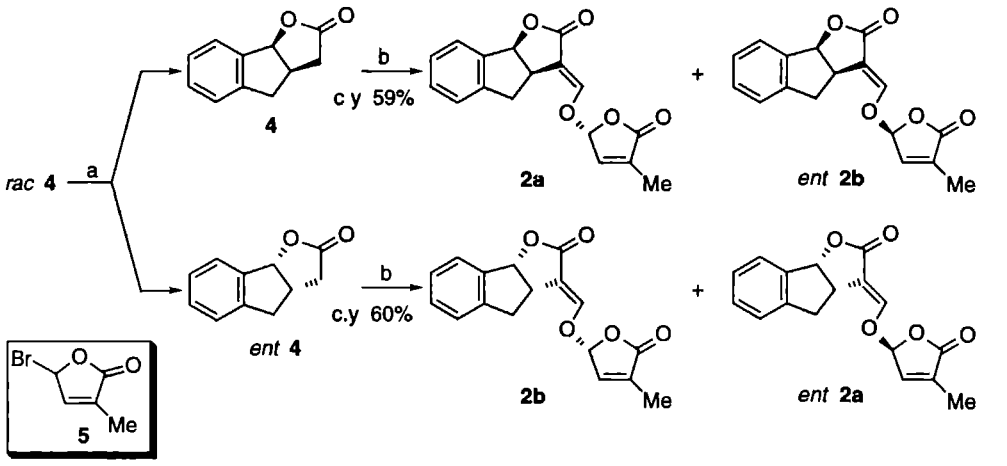
It is noteworthy that all strigolactones as well as the highly potent synthetic analogues GR24 and GR7 have identical D-rings, which is therefore an attractive target to introduce optical activity. An important achievement in obtaining these enantiopure analogs is the successful resolution of a common D-ring precursor as is outlined in chapters 2 and 3. This chapter deals with the asymmetric synthesis of all four stereoisomers of GR24 (**2**) by making use of this newly developed concept as well as another independent route. In addition a comparative study regarding their germination stimulatory activities toward seeds of *Striga hermonthica* (Del.) Benth and *Orobanche crenata* Forsk. is described.

5.2 Results and discussion

Synthesis

In order to achieve the resolution of GR24 (**2**), two synthetic approaches were considered. The first approach (scheme 1) involves the separation of tricyclic lactone *rac.* **4** into its enantiomers, followed by formylation and coupling of the individual enantiomers with racemic 5-bromofuranone **5** in an analogous manner as described previously for the racemic preparation of GR24.[10] Tricyclic lactone *rac.* **4a** was chromatographically resolved using cellulose triacetate (CTA) as the chiral stationary phase. It has been demonstrated that a wide variety of racemic γ- and δ-lactones can be separated by column chromatography on the chiral phase CTA, both on an analytical and preparative scale.[16] In the present case 500 mg of *rac.* **4** was resolved by flash-chromatography at a pressure of ca. 1.2 bar, using 50 g of CTA and 95% ethanol as the eluent, in a total recovery of enantiopure tricyclic lactones **4** and *ent.* **4** of 68%. The enantiopurity of **4** and *ent.* **4** was assessed by HPLC, using cellulose carbamate as the chiral stationary phase. The diastereomeric mixtures obtained after formylation and coupling of **4** and *ent.* **4** with **5** (scheme 1) were both separated by flash-chromatography (SiO_2) to give **2a**, *ent.* **2b** and **2b**, *ent.* **2a** (e.e.'s > 98%) in total isolated yields of 59% and 60%, respectively. The e.e.'s were determined by ^{1}H-NMR using the chiral shift reagent Eu(hfc)$_3$.

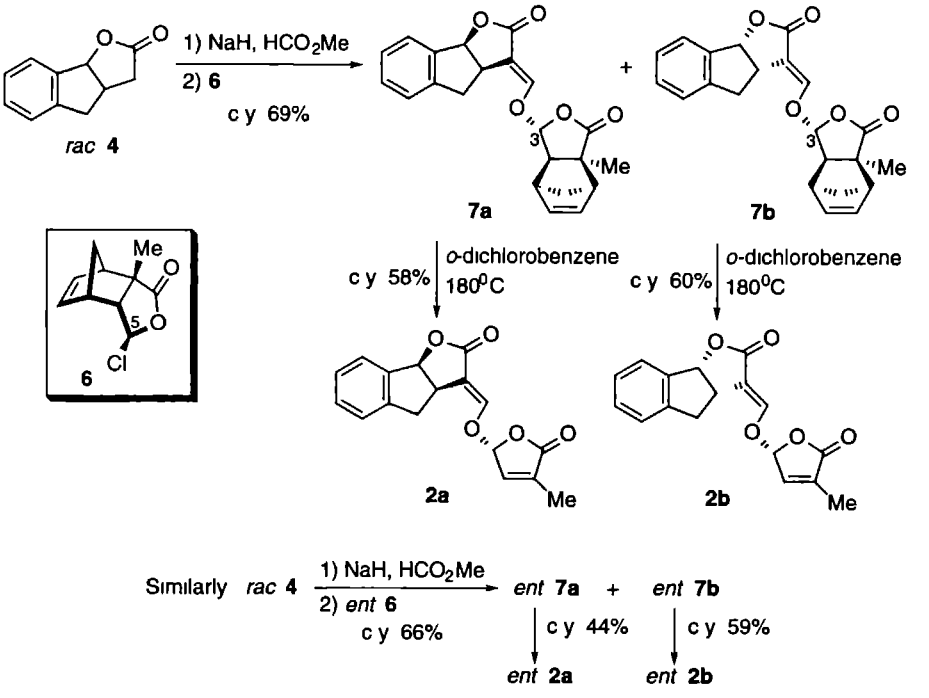
Scheme 1



a) cellulose tnacetate chromatography, b) 1 KO^tBu, HCO_2Me, 2 **5**

In the second approach the complete resolution of GR24 **2** was accomplished by formylation of *rac* **4**, followed by coupling with the homochiral latent D-rings **6** and *ent* **6**, respectively (scheme 2)

Scheme 2



The asymmetric syntheses of **6** and *ent.* **6**, together with their application in the preparation of all four homochiral diastereomers of GR7 (**3**), has recently been described.[17] The sequence as is outlined in scheme 2 gave the cycloadducts **7a** and **7b** and their corresponding enantiomers in diastereomeric ratios of approximately 1:1. These reactions proceed with complete stereocontrol as no signals arising from the respective C-3-epimers could be detected in the NMR-spectra. Cycloreversion by heating the homochiral adducts of **7** in *o*-dichlorobenzene at 180^{0}C afforded the corresponding enantiopure stereoisomers of GR24 (**2**), which have the same $[\alpha]_D$-values as those prepared by the route depicted in scheme 1. It was essential to control the reaction temperature and time carefully, in order to avoid concomitant epimerization at C-2' of **2**. This epimerization most likely occurs via tautomerization of the vinylogous lactone function to give the corresponding achiral 2-hydroxy furan moiety as an intermediate.[18]

Determination of the absolute configuration

It was attempted to establish the absolute configuration of the GR24 (**2**) stereoisomers by comparison of their circular dichroism (CD) spectra with those of the corresponding stereoisomers of strigol **1**, which have been reported.[19,20] However, such a correlation is not reliable, because GR24 (**2**) has a different ABC-chromophore. Therefore, an X-ray diffraction analysis of **2a** was undertaken[21] to establish its absolute configuration. A stereoview of **2a** is depicted in fig 2. Knowing the absolute configuration of **2a**, the configuration of the remaining stereoisomers could be assigned on the basis of the synthetic sequence.

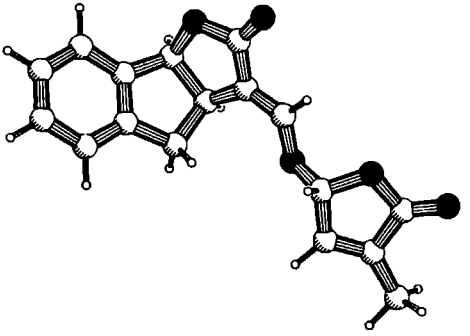


Figure 2. PLUTON generated drawing of X-ray crystal structure of **2a**

Biological Activity

The germination stimulatory activity of all stereoisomers of GR24 **2a,b** and *ent.* **2a,b** was assayed using seeds of *Striga hermonthica* and *Orobanche crenata*. In each bioassay a diastereomeric mixture of GR24 was included as a positive control. In preliminary experiments the concentration dependent activity range (GR24) of seeds of *Striga hermonthica* has been established. Maximal germination percentages were obtained within the concentration range of 1 mg/L and 0.01 mg/L. Half-maximal activity was observed at approximately 0.001 mg/L (data not

shown). The relative bioactivity of the individual stereoisomers of GR24 was therefore assayed at an optimal concentration (0.1 mg/L) and at a sensitive concentration (0.001 mg/L). It was anticipated that the latter should exhibit more profound differences. Relevant data are collected in Table I.

Table I. Germination percentages for seeds of *Striga hermonthica* after exposure to solutions (0.1 and 0.001 mg/L) of GR24 enantiomers **2**, and the corresponding racemic mixture of diastereomers *rac.* **2**. The data presented ± S.E are from one representative experiment.

entry	compound	configuration at C-2'	Concentration (mg/L) 10^{-1}	10^{-3}
			% germination ± S.E.	
1	**2a**	R	56.2 ± 4.6	32.5 ± 4.5
2	*ent.* **2b**	S	40.8 ± 2.5	0.7 ± 0.4[b]
3	*ent.* **2a**	S	4.0 ± 0.6	0.5 ± 0.5[b]
4	**2b**	R	54.0 ± 2.6	0.4 ± 0.4[b]
5	*rac.* **2**[a]	R/S	47.1 ± 3.9	33.2 ± 2.2

a) Equimolar mixture of two racemic diastereomers
b) Not significantly different from aqueous control (without stimulant)

The same stereoisomers were also tested for stimulant activity on seeds of *Orobanche ıta*, using four concentrations. The results are shown in fig. 3.

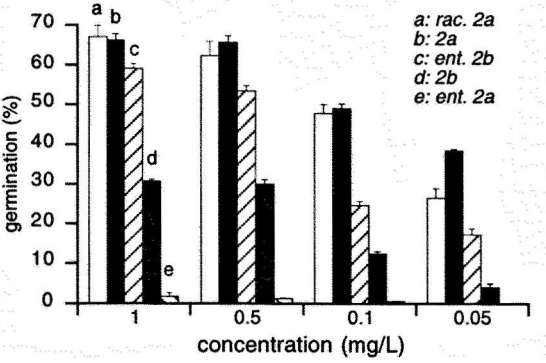


Figure 3. Germination percentages for seeds of *Orobanche crenata* after exposure to different concentrations of GR24 enantiomers **2**. The data presented ± S.E. are from one single representative experiment.

These data (Table I, fig. 3) reveal that there is a significant difference in stimulatory activity between the four stereoisomers. For both parasitic species, the enantiomer of GR24 possessing the "natural" absolute stereochemistry, *viz.* **2a**, is considerably more active than its optical

antipode *ent.* **2a**. The difference in activity amounts at least a factor of 100. The relative importance of the different stereogenic centers can be adressed by comparison of the bioactivities of the C-3a, C-8b-epimer **2b** and the C-2'-epimer *ent.* **2b**. The data in Table I (entries 2 and 4) reveal in the case of *Striga hermonthica* a slight preference for the correct stereochemistry at C-2'. In contrast, the data obtained for *Orobanche crenata* (fig. 3) suggest that for this species the absolute configuration at C-3a, C-8b is more important for the stimulatory activity than the configuration at C-2'. This result is in contrast with data obtained from a comparative study of GR7 stereoisomers, which revealed a more profound role of the C-2'-configuration.[15] This discrepancy indicates that it is not allowed to attribute the configuration of one particular stereogenic center as solely relevant for the biological activity, by disregarding the entire three-dimensional structure. Because GR7 lacks the A-ring, its smaller BC-part is apparently more flexible with respect to interactions within the receptor cavity. This implies a less critical requirement for its absolute configuration in comparison with the ABC-fragment of GR24.

5.3 Concluding remarks

Two routes for the asymmetric synthesis of all four stereoisomers of GR24 have been presented. The route which requires the homochiral latent D-rings **6** and *ent.* **6** is generally applicable in the enantioselective synthesis of strigol analogues. The relative importance of the absolute configuration at the different stereogenic centers in GR24 for the germination stimulatory activity have been assessed, indicating that the molecular shape is determined by the combination of all stereocenters.

5.4 Experimental section

Synthesis

General Remarks

For general methods and instrumentation, see ref.[17] 3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one *rac.* **4** and 5-bromo-3-methyl-2(5H)-furanone **5** were prepared following published methods.[10] The synthesis of chloro lactones **6** and *ent.* **6** was reported previously.[17]

For the resolution of *rac.* **4** microcrystalline cellulose triacetate (Fluka, particle size 25-40 μm) was used. The enantiopurities of **4** and *ent.* **4** were determined by HPLC analysis using an Spectra Physics SP8700 HPLC apparatus, a chiral Baker CHIRALCEL OD-H column (eluent hexane/ethanol 60:40 (v/v), flow rate 0.5 mL/min) and an LKB 2138 Uvicord S UV/VIS detector (254 nm).

Chromatographic resolution of 3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (rac. **4** *)*

The sorbent CTA (50 g) was swollen before use by heating in EtOH/H_2O 95:5 (150 mL) at ca. 75°C for 20 min. A glass column (i.d. 2.5 cm) was slurry-packed with this material and the stationary phase was eluted with 95% EtOH at a pressure of ca. 1.2 bar. The racemic lactone *rac.* **4**, dissolved in 95% EtOH (3 mL), was chromatographically resolved, using 95% EtOH as the

eluent. Fractions of ca. 5 mL were collected, their optical rotations were recorded, and analyzed for e.e. by chiral HPLC.
Yield 170 mg (34%) of pure fast-moving enantiomer **4** (t_R 11.30 min.) as a white solid. $[\alpha]_D$ -107.0^0 (c 0.4, $CHCl_3$), e.e. > 98%.
The slow-moving enantiomer *ent.* **4** (170 mg, 34%) (t_R 12.18 min.) was obtained as a white solid. $[\alpha]_D$ $+102.5^0$ (c 0.4, $CHCl_3$), e.e. > 98%.

Formylation of 3a(R),8b(S)-4 and coupling with 5-bromo-3-methyl-2(5H)-furanone 5 (general procedure)
Potassium *tert*-butoxide (131 mg, 1.17 mmol) was added to a solution of lactone **4** (185 mg, 1.06 mmol) and methyl formate (1.6 mmol) in THF (5 mL) with stirring at 0^0C under nitrogen. Stirring was continued for 18h at room temperature, followed by the addition of a solution of furanone **5** (207 mg, 1.17 mmol) in THF (10 mL) at 0^0C under nitrogen. After stirring for 18h, precipitated potassium bromide was removed by filtration. The filtrate was concentrated *in vacuo*, and the residue was dissolved in a mixture of water and chloroform. The aqueous phase was extracted with chloroform (3x). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude yellow oil was purified by flash chromatography (SiO_2, di*iso*propyl ether, ethyl acetate 4:1) to give two separated diastereomeric products **2a** and *ent.* **2b** as white solids.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene)-3,3a(R),4,8b(S)-tetrahydro-indeno[1,2-b]furan-2-one (**2a**) *and its 2'(S)-epimer* (*ent.* **2b**)
These compounds were prepared according to the general procedure to give **2a** (yield 29%) and *ent.* **2 b** (yield 30%). Analytically pure samples were obtained by crystallization from hexane/ethyl acetate.
2a. Obtained as colorless crystals. Mp 154-155^0C; $[\alpha]_D$ $+436^0$ (c 0.25, $CHCl_3$); R_f 0.32 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.05 (m, 3H, C$\underline{H}_3$), 3.11 (dd, 1H, ^{2}J 16.8 Hz, J 3.3 Hz, $\underline{H}_4$), 3.44 (dd, 1H, ^{2}J 16.8 Hz, J 9.2 Hz, $\underline{H}_4$), 3.95 (m, 1H, $\underline{H}_{3a}$), 5.96 (d, 1H, J 7.8 Hz, $\underline{H}_{8b}$), 6.18 (m, 1H, OC$\underline{H}$O D-ring), 6.97 (m, 1H, =C$\underline{H}$ D-ring), 7.23-7.36 (m, 3H, Ar $\underline{H}$), 7.48 (d, 1H, ^{4}J 2.5 Hz, =C$\underline{H}$O), 7.51 (d, 1H, J 7.1 Hz, Ar $\underline{H}$) ppm; MS (EI, m/z, rel. int. (%)): 298 ([M]$^+$, 0.4), 201 ([$C_{12}H_9O_3$]$^+$, 39.3), 97 ([$C_5H_5O_2$]$^+$, 100); Analysis calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.22; H, 4.63.
ent. **2b**. Obtained as colorless crystals. Mp 133.5-134.5^0C; $[\alpha]_D$ $+273^0$ (c 0.2, $CHCl_3$); R_f 0.24 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.05 (m, 3H, C$\underline{H}_3$), 3.10 (dd, 1H, ^{2}J 16.9 Hz, J 3.1 Hz, $\underline{H}_4$), 3.42 (dd, 1H, ^{2}J 16.9 Hz, J 9.3 Hz, $\underline{H}_4$), 3.94 (m, 1H, $\underline{H}_{3a}$), 5.96 (d, 1H, J 7.9 Hz, $\underline{H}_{8b}$), 6.17 (m, 1H, OC$\underline{H}$O D-ring), 6.96 (m, 1H, =C$\underline{H}$ D-ring), 7.23-7.36 (m, 3H, Ar $\underline{H}$), 7.48 (d, 1H, ^{4}J 2.5 Hz, =C$\underline{H}$O), 7.50 (d, 1H, J 7.5 Hz, Ar $\underline{H}$) ppm; Mass data were the same as for **2a**. Analysis calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.31; H, 4.68.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene)-3,3a(S),4,8b(R)-tetrahydro-indeno[1,2-b]furan-2-one (**2b**) *and its 2'(S)-epimer* (*ent.* **2a**)
These compounds were prepared according to the general procedure, starting from lacone *ent.* **4** (186 mg, 1.07 mmol) to give **2b** (yield 33%) and *ent.* **2a** (yield 27%). Analytically pure samples were obtained by crystallization from hexane/ethyl acetate.

2b. Obtained as colorless crystals. Mp 133.5-134^{0}C; $[\alpha]_D$ -272^0 (c 0.2, $CHCl_3$); Analysis calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.26; H, 4.66. ^{1}H-NMR and mass data were the same as for compound *ent.* **2b**.
ent. **2a**. Obtained as colorless crystals. Mp 152.5-154.5^{0}C; $[\alpha]_D$ -446^0 (c 0.25, $CHCl_3$); Analysis calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.12; H, 4.67. ^{1}H-NMR and mass data were the same as for compound **2a**.

Determination of enantiopurities
400 MHz ^{1}H-NMR analysis of racemic mixtures of diastereomers **2a** and **2b** with optical shift reagent Eu(hfc)$_3$ (0.5 eq.) revealed a downfield shift for all resonances, with a prominent 1:1 splitting of the signal for the enol ether proton (=C<u>H</u>-O), amounting 0.35 ppm. 400 MHz ^{1}H-NMR spectra of pure compounds **2a,b** and *ent.* **2a,b** displayed comparable shifts on treatment with Eu(hfc)$_3$, but in contrast to the racemates, no splitting of signals was observed, indicating an enantiopurity of at least 98%.

3-(6(S)-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]dec-8-en-3(R)-yloxymethylene)-3,3a(R),4,8b(S)-tetrahydro-indeno[1,2-b]furan-2-one (**7a**) *and its 3a(S),8b(R) diastereomer* (**7b**)
To a stirred suspension of NaH (139 mg, 3.48 mmol) in diethyl ether (10 mL) a solution of *rac.* **4** (300 mg, 1.72 mmol) in diethyl ether (5 mL) was gradually added at room temperature under nitrogen. Ethyl formate (1.7 mL, 21 mmol) was added and stirring was continued for 15 h. The solvent was removed *in vacuo*. The thus obtained sodium salt was dissolved in DMF (10 mL). A solution of chloro lactone **6** (372 mg, 1.87 mmol) in DMF (3 mL) was gradually added at room temperature under nitrogen. After 17 h of stirring the mixture was quenched with acetic acid (0.5 mL) and the solvent was removed *in vacuo*. The residue was dissolved in a mixture of water and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2x) and the combined organic layers were washed with water (2x), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified using flash chromatography (SiO_2, hexane / ethyl acetate 2:1) to afford two diastereomeric products. The fast moving diastereomer **7a** (222 mg, 35%) was obtained as a white solid, and crystallization from di*iso*propyl ether/ ethyl acetate afforded analytically pure **7a**. The slow moving diastereomer **7b** (211 mg, 34%) was obtained as a white solid, which gave an analytically pure sample after crystallization from hexane/ ethyl acetate.
7a. Mp 174-177^{0}C; $[\alpha]_D$ +276^0 (c 0.1, $CHCl_3$); R_f 0.48 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.61 (s, 3H, C<u>H</u>$_3$), 1.74 (m, 2H, <u>H</u>$_{10}$), 2.74 (dd, 1H, J 4.2 Hz, J <1 Hz, <u>H</u>$_2$), 2.91 (m, 1H, <u>H</u>$_7$), 3.09 (dd, 1H, ^{2}J 16.8 Hz, J 3.8 Hz, C<u>H</u>$_2$ B-ring), 3.24 (m, 1H, <u>H</u>$_1$), 3.46 (dd, 1H, ^{2}J 16.8 Hz, J 8.9 Hz, C<u>H</u>$_2$ B-ring), 3.93 (m, 1H, <u>H</u>$_{3a}$), 5.23 (d, 1H, J <1 Hz, <u>H</u>$_3$), 5.94 (d, 1H, J 7.8 Hz, <u>H</u>$_{8b}$), 6.25 (m, 2H, <u>H</u>$_8$ and <u>H</u>$_9$), 7.27-7.55 (m, 4H, Ar <u>H</u>), 7.40 (d, 1H, ^{4}J 2.5 Hz, =C<u>H</u>O) ppm; MS (EI, m/z, rel. int. (%)):364 ([M]$^+$, 0.3), 299 ([$C_{17}H_{15}O_5$]$^+$, 0.3), 203 ([$C_{12}H_{11}O_3$]$^+$, 27.1), 163 ([$C_{10}H_{11}O_2$]$^+$, 93.2), 97 ([$C_5H_5O_2$]$^+$, 100), 66 ([C_5H_6]$^+$, 15.6); Analysis calcd for $C_{22}H_{20}O_5$: C, 72.52; H, 5.53. Found: C, 72.11; H, 5.46.
7b. Mp 192.5-194.5^{0}C; $[\alpha]_D$ -334^0 (c 0.1, $CHCl_3$); R_f 0.36 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.63 (s, 3H, C<u>H</u>$_3$), 1.73 (m, 2H, <u>H</u>$_{10}$), 2.78 (dd, 1H, J 3.7 Hz, J <1 Hz, <u>H</u>$_2$), 2.91 (m, 1H, <u>H</u>$_7$), 3.05 (dd, 1H, ^{2}J 16.8 Hz, J 3.5 Hz, C<u>H</u>$_2$ B-ring), 3.24 (m, 1H, <u>H</u>$_1$), 3.42 (dd, 1H, ^{2}J 16.8 Hz, J 8.8 Hz, C<u>H</u>$_2$ B-ring), 3.93 (m, 1H, <u>H</u>$_{3a}$), 5.24 (d, 1H, J <1 Hz, <u>H</u>$_3$), 5.95 (d, 1H, J 7.9 Hz, <u>H</u>$_{8b}$), 6.26 (m, 2H, <u>H</u>$_8$ and <u>H</u>$_9$), 7.21-7.53 (m, 4H, Ar <u>H</u>), 7.39 (d, 1H, ^{4}J 2.8 Hz, =C<u>H</u>O) ppm; Mass data were the same as for **7a**. Analysis calcd for $C_{22}H_{20}O_5$: C, 72.52; H, 5.53. Found: C, 71.91; H, 5.47.

3-(6(R)-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]dec-8-en-3(S)-yloxymethylene)-3,3a(R),4,8b(S)-tetrahydro-indeno[1,2-b]furan-2-one (*ent.* **7b**) *and its 3a(S),8b(R) diastereomer* (*ent.* **7a**)
These compounds were prepared in the same way as described for **7a** and **7b**, starting from *rac.* **4** (302 mg, 1.73 mmol) and chloro lactone *ent.* **6** (379 mg, 1.91 mmol). The fast moving diastereomer *ent.* **7a** (202 mg, 32%) was obtained as a white solid, and crystallization from diisopropyl ether/ ethyl acetate afforded analytically pure *ent.* **7a**. The slow moving diastereomer *ent.* **7b** (215 mg, 34%) was obtained as a white solid, which gave an analytically pure sample after crystallization from hexane/ ethyl acetate.
ent. **7a**. Mp 171-173.5^{0}C; $[\alpha]_D$ -278^0 (c 0.1, $CHCl_3$); Analysis calcd for $C_{22}H_{20}O_5$: C, 72.52; H, 5.53. Found: C, 72.58; H, 5.45. ^{1}H-NMR and mass data were the same as for compound **7a**.
ent. **7b**. Mp 193-193.5^{0}C; $[\alpha]_D$ +340^0 (c 0.1, $CHCl_3$); Analysis calcd for $C_{22}H_{20}O_5$: C, 72.52; H, 5.53. Found: C, 72.17; H, 5.53. ^{1}H-NMR and mass data were the same as for compound **7b**.

Cycloreversion of adducts 7a,b and ent. 7a,b
A solution of the enantiopure cycloadduct **7** (93 mg, 0.26 mmol) in *o*-dichlorobenzene (40 mL) was heated at 180^{0}C for 14 h. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, hexane/ ethyl acetate 2:1) to give the corresponding enantiomer of **2** (44 mg, 58%) as a solid. All enantiomers of **2**, prepared via this procedure showed identical physical and chiroptical properties as described above.

Biological activity
Seeds
Seeds of *Striga hermonthica* (from *Sorghum bicolor* (L.) Moench) and *Orobanche crenata* (from *Vicia faba* L.) were harvested in Burkina Faso in 1994 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests. Bioassays were carried out essentially following the procedure of Mangnus *et al.*[14] with minor modifications.
Preparation of test solutions
A compound to be tested was weighed out very accurately to the amount of 2.5 mg, dissolved in 5 mL of acetone p.a. and diluted with demineralized water to 25 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0.1, and 0.01 mg/L test compound and 0.2, 0.02, and 0.002% (v/v) acetone, respectively.
Bioassays
For surface sterilization seeds of *Striga hermonthica* were subsequently exposed to 70% (v/v) ethanol for 5 min. and sodium hypochlorite (2% active chlorine) for 2 min with agitation. Seeds of *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried.
For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30-70 seeds per disk) in Petri dishes, each containing 2 disks (*Striga*) or 4 disks (*Orobanche*), wetted with water, and stored in the dark for 14 days at 20^{0}C for *Orobanche* seeds and at 30^{0}C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk (*Orobanche*) or 3 mL per Petri dish (*Striga*). After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at the indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.2, 0.02 and 0.002% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (concentrations of 1, 0.1 and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 6 disks (*Striga*) or 12 disks (*Orobanche*) per treatment.

5.5. References

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Chapter 6*

Asymmetric Synthesis of All Stereoisomers of Desmethyl Sorgolactone. Dependence of the Absolute Configuration on the Stimulatory Activity of *Striga hermonthica* and *Orobanche crenata* Seed Germination

Abstract: Strigol and sorgolactone belong to the class of "strigolactones", which are highly potent germination stimulants of seeds of the parasitic weeds *Striga* and *Orobanche* Two diastereomers of desmethyl sorgolactone **6**, which lacks the methyl group in the A-ring of sorgolactone, were prepared and resolved in the corresponding enantiomers. Bioassays revealed that the germination stimulatory activity of **6** is comparable to that of strigol and that their exist significant differences in activity between the individual stereoisomers.

6.1 Introduction

After the identification of (+)-strigol (**1**) as a naturally occurring seed germination stimulant of the parasitic weeds *Striga* and *Orobanche*,[1] isolated from the root exudate of the false host cotton (*Gossypium hirsutum* L),[2] several total syntheses of this extremely active compound have been reported A complete list of publications covering this synthetic work is given in chapter 1 The related structures **2** (sorgolactone)[3] and **3** (alectrol)[4] are the first characterized strigol analogs that were isolated from true *Striga* hosts Recently, the collective name 'strigolactones' was proposed for the naturally occurring compounds **1-3** [5] So far, no germination stimulants from *Orobanche* hosts have been identified However, it is assumed that structures closely related to the strigolactones are primarily responsible for germination stimulation of *Orobanche* seeds under natural conditions It should be noted that the assignment of structures **2** and **3** is tentatively based on spectroscopic evidence, as they were obtained in minute amounts and in an impure form So far, no independent synthesis of either sorgolactone or alectrol has been reported to assure their proposed structures by comparison of the spectroscopic data An important aspect in the synthesis of **2** and **3** will be the control of the stereochemistry Recently, we achieved full control of the absolute configuration of the D-ring at C-2', as is demonstrated by the synthesis of all stereoisomers of the synthetic strigol analogs GR24 (**4**) and GR7 (**5**) Details are described in chapters 5 and 2, respectively

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This chapter is devoted to the asymmetric synthesis of a model compound for sorgolactone, *viz.* desmethyl sorgolactone **6** (DMSL). By omitting the methyl substituent in the A-ring the stereochemical difficulties are considerably reduced. For the introduction of a specific stereochemistry in the D-ring the same tactic as applied in the synthesis of GR24[6] and GR7[7] is followed. Besides the synthetic aspects an evaluation of the germination stimulatory activities of all four single stereoisomers of DMSL (**6**) toward seeds of *Striga hermonthica* (Del.) Benth. and *Orobanche crenata* Forsk. is described.

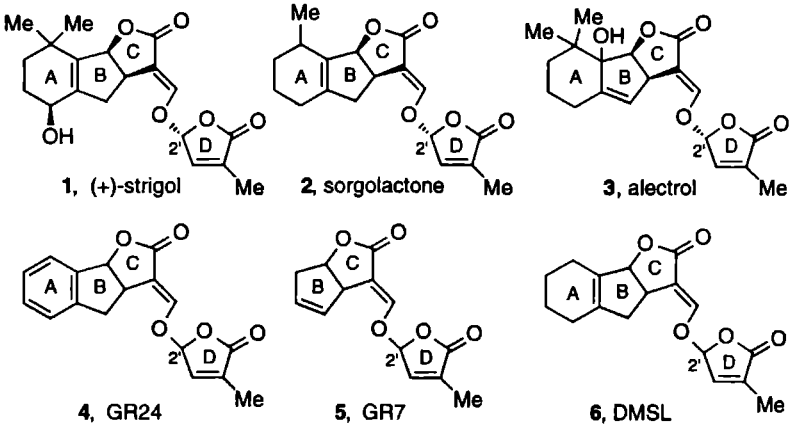


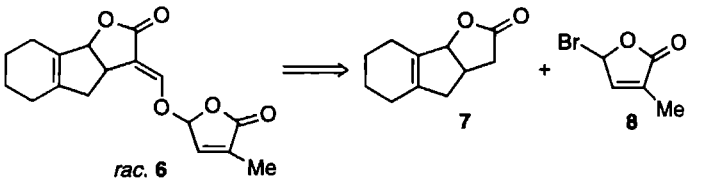
Figure 1

6.2 Results and discussion

Synthesis of racemic DMSL

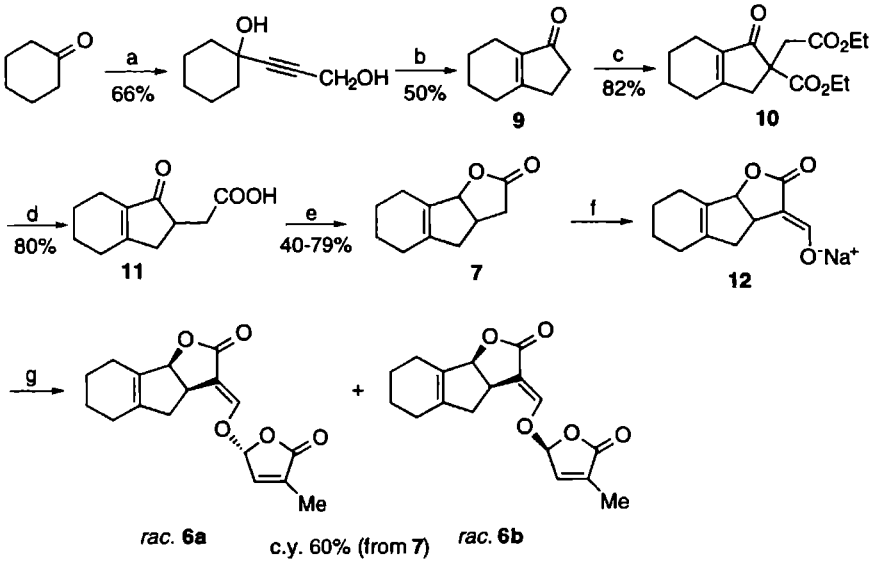
Retrosynthetic analysis of DMSL (*rac.* **6**) leads to the key building blocks **7** and **8** (scheme 1), which are coupled in the final step via an vinyl ether linkage.

Scheme 1



This strategy resembles that used for the synthesis of strigol and its analogues.[8] Tricyclic lactone **7** was prepared using essentially the concept (scheme 2) for the synthesis of the ABC-fragment of strigol.[9,10]

Scheme 2



a) propargyl alcohol (1.5 equiv.), *n*-BuLi (3.3. equiv.) b) MeOH, H_2SO_4
c) 1. NaH (2 equiv.), $(EtO)_2CO$ 2. $BrCH_2CO_2Et$ d) HOAc, HCl
e) DIBALH or $NaBH_4/CeCl_3.7H_2O$ f) NaH, HCO_2Et g) **8**

Bicyclopentenone **9** was prepared in two steps from cyclohexanone via addition of the dianion of propargyl alcohol, followed by an acid-induced *in situ* Rupe rearrangement and Nazarov-type electrocyclization of the pentadienyl cation intermediate.[11] Carboxylic acid **11** was obtained by a procedure, analogous to that described for GR24 (**4**).[8] Attempted reduction of the ketone function by alkaline $NaBH_4$ to obtain **7** was not successful. The preferred reaction course was 1,4-reduction under these conditions. However, DIBALH treatment afforded the desired 1,2-reduction in a stereoselective fashion to give tricyclic lactone **7** in yields ranging from 40-64%. Better results were obtained using $NaBH_4$ (4 equiv.) in the presence of $CeCl_3.7H_2O$ (2 equiv.),[12] which gave **7** in a reproducible yield of 79%. Coupling of **7** via formylation and subsequent reaction of the intermediate sodium enolate **12** with bromofuranone **8**[8] provided DMSL **6** as a mixture of diastereomers *rac.* **6a** and *rac.* **6b**, which could readily be separated by chromatography on silica gel (scheme 2).

Asymmetric synthesis of DMSL

Recently, the synthesis of the homochiral latent D-ring synthons, *viz.* **13** and *ent.* **13** (fig 2), has been reported.[7] Tricyclic lactone **7** was coupled via its sodium enolate **12** with chloro lactones **13** or *ent.* **13** to give diastereomeric mixtures (ratio ca. 1:1) of **14a**, *ent.* **14b** (c.y. 82%) and **14b**, *ent.* **14a** (62%), respectively (fig. 2), which were separated by flash-chromatography.

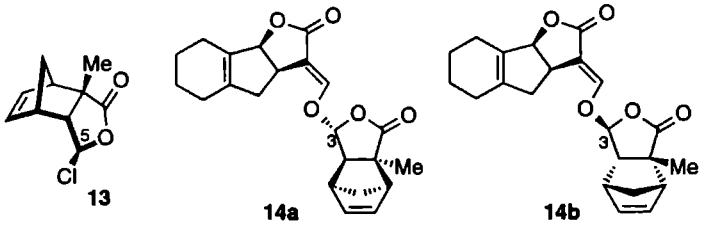
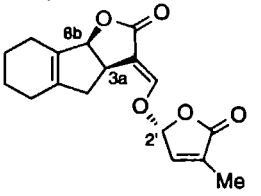


Figure 2

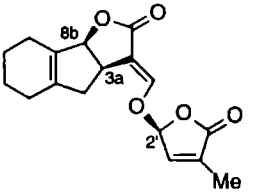
These reactions were carried out in DMF as the solvent and proceed with complete *exo*-selectivity as was deduced from the observed ^{3}J-coupling constants between H_3 and H_2 in the ^{1}H-NMR spectrum, (*cf* ref. 7).

The thermal retro Diels-Alder reaction of homochiral adducts **14a,b** and *ent.* **14a,b** to give the corresponding enantiopure DMSL stereoisomers **6a,b** and *ent.* **6a,b**, respectively, was accomplished by heating these adducts in *o*-dichlorobenzene at 180^0. It is essential to control the reaction time and temperature carefully, since the stereocenter at C-2' is rather sensitive to epimerization. The results obtained for the cycloreversion are collected in Table I. It should be noted that in none of these reactions any epimerization was observed.

Table I. Cycloreversion of adducts **14a,b** and their enantiomers



6a, 3a(R),8b(S),2'(R)



6b, 3a(R),8b(S),2'(S)

entry	adduct	product	yield (%)	$[\alpha]_D^a$
1	**14a**	**6a**	30	+281^0
2	**14b**	**6b**	42	+160^0
3	*ent.* **14a**	*ent.* **6a**	36	-285^0
4	*ent.* **14b**	*ent.* **6b**	31	-150^0

a) $[\alpha]_D$ of products after cycloreversion. For details, see the experimental section

The enantiopurity of DMSL stereoisomers **6a,b** and *ent.* **6a,b** (Table I) was determined by 400 MHz ^{1}H-NMR analysis using chiral shift reagent Eu(hfc)$_3$. The spectra obtained were compared with those of the corresponding racemic mixtures under the same circumstances. All compounds **6a,b** and *ent.* **6a,b** have an enantiopurity of >98%; in no case signals of the antipode could be detected. It is thus demonstrated that this asymmetric synthesis affords excellent stereocontrol at C-2' of the D-ring. It should be noted that our strategy which involves the use of a

homochiral latent D-ring precursor has a much larger scope than the previously reported procedures.[13-17] The latter all comprise asymmetric synthesis of a particular ABC-precursor.

Determination of absolute configuration

The correct absolute configurations of DMSL enantiomers **6a**, *ent.* **6a**, **6b** and *ent.* **6b** were established by comparison of their circular dichroism (CD) spectra with those of the corresponding stereoisomers of strigol. The CD-spectra of (+)- and (-)-strigol have been reported.[14] More recently, Frischmuth *et al.* compared the CD-curves of (+)- and (-)-strigol with those of their respective 2'-epimers.[18] It was concluded that the sign of the Cotton-effect at 270 nm could directly be correlated with the stereochemistry at C-2', a negative CD-sign corresponding with the 2'(R) configuration. Hauck *et al.* observed that the CD-spectrum of sorgolactone **2** is identical with that of naturally occurring (+)-strigol and inverse to the spectrum of the antipode (-)-strigol.[3] On the basis of these data, it is justified to assign the absolute stereochemistry of the DMSL enantiomers **6** using their CD-curves. These spectra, which are depicted in fig 3 are nearly identical with those of the respective stereoisomers of strigol.[18]

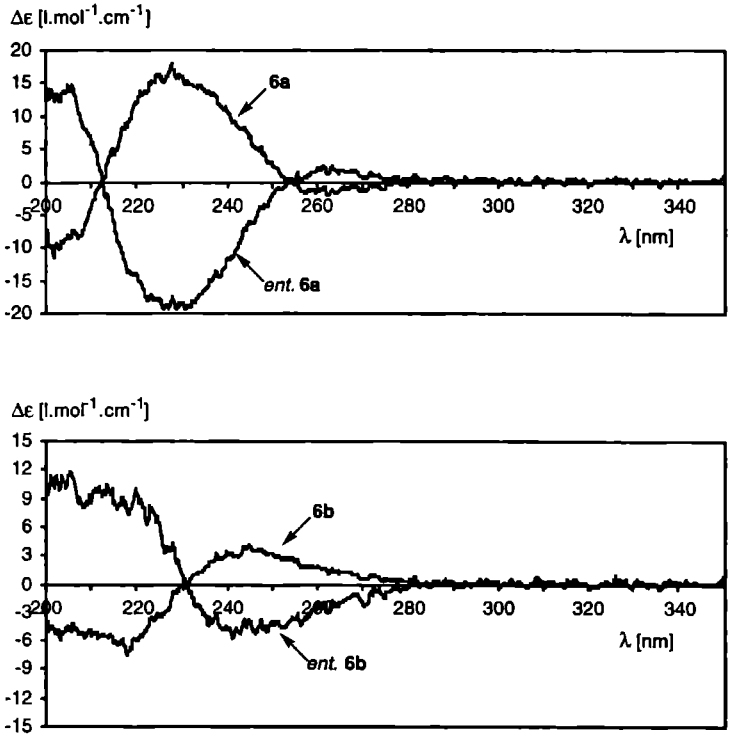


Figure 3. CD-Spectra of DMSL stereoisomers **6a** (c 19.8 μM), **6b** (c 24.8 μM), *ent.* **6a** (c 19.8 μM), and *ent.* **6b** (c 29.8 μM) using acetonitrile as the solvent

The configuration at C-2', as deduced from the CD-sign around 270 nm, is in all four cases in complete agreement with the expected stereochemistry, based on the chirality of the latent D-ring synthon (fig. 2). The stereochemistry of the ABC-part was assigned (Table I) by comparison of the shape of the CD-curves with that of the corresponding stereoisomers of strigol.

Biological Activity.

The germination stimulatory activity of all stereoisomers of DMSL (**6a,b** and *ent.* **6a,b**) was assayed using seeds of *Striga hermonthica* and *Orobanche crenata*. In each bioassay diastereomeric mixtures of GR24 and DMSL were included as positive controls. In preliminary experiments the concentration dependent activity range (GR24 and DMSL) of seeds of *Striga hermonthica* has been established. Maximal germination percentages were obtained within the concentration range of 1 mg/L and 0.01 mg/L. Half-maximal activity was observed at approximately 0.001 mg/L (data not shown). Assessment of the relative bioactivity of the individual stereoisomers of DMSL was therefore established at an optimal concentration (0.1 mg/L) and at a sensitive concentration (0.001 mg/L). It was anticipated that the lower concentration should exhibit more profound differences. Relevant data are collected in Table II. The same compounds were also tested for stimulant activity on *Orobanche crenata* seeds at three concentrations, the results of which are shown in fig. 4.

Table II. Germination percentages for seeds of *Striga hermonthica* after exposure to solutions (0.1 and 0.001 mg/L) of DMSL enantiomers **6** and the corresponding diasteromeric mixture[a]

entry	compound	configuration at C-2'	Concentration (mg/L) 10^{-1}	10^{-3}
			% germination ± S.E.	
1	**6a**	R	63.2 ± 2.6	42.5 ± 1.2
2	**6b**	S	61.0 ± 3.5	0.0 ± 0.0[c]
3	*ent.* **6a**	S	22.8 ± 4.6	0.7 ± 0.7[c]
4	*ent.* **6b**	R	56.3 ± 4.8	1.6 ± 0.8
5	*rac.* **4**[b]	R/S	47.1 ± 3.9	33.2 ± 2.2
6	*rac.* **6**[b]	R/S	56.0 ± 1.0	28.3 ± 7.4

a) Data presented are the mean ± S.E. of one representative experiment
b) Equimolar mixture of two racemic diastereomers
c) Not significantly different from aqueous control (without stimulant)

Rac. GR24 and *rac.* DMSL exhibit similar bioactivities for seeds of *Striga hermonthica* (entries 5 and 6, Table II) and *Orobanche crenata* (fig. 4). It should be added that none of these compounds showed stimulatory activity toward seeds of the related root parasite *Striga gesnerioides* (data not shown).

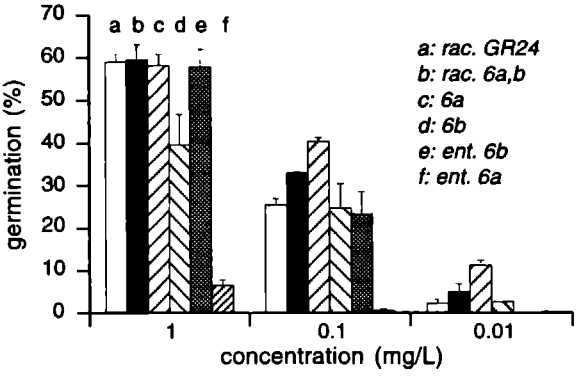


Figure 4. Germination percentages for seeds of *Orobanche crenata* after exposure to different concentrations of DMSL stereoisomers **6**. The data presented are the mean ± S.E. of three replicate tests.

Thus, replacement of the aromatic A-ring present in GR24 by a cyclohexene fragment has only a marginal effect on the respective bioactivities. This conclusion is in agreement with previously performed comparative studies of strigol and its synthetic analogues. Hauck *et al.* found an activity of (+)-strigol on seeds of *Striga hermonthica* of 10^{-9}M (concentration at half-maximal activity), which is almost the same as that of GR24 (**4**).[3] Similarly, (+)-strigol and *rac.* **4** are almost equally active (half-maximal activity at 10^{-7}M) toward stimulation of seed germination of *Orobanche crenata.*[19] Pepperman has reviewed the biological activity of strigol and its analogues with respect to germination of seeds of several parasitic weed species.[20] It was concluded that the activity of strigol is comparable to that of GR24.

Next, the influence of the stereochemistry on the bioactivities of DMSL enantiomers **6a,b** and *ent.* **6a,b** was examined. As expected, **6a**, which possesses the "natural" absolute stereochemistry, is considerably more active than its optical antipode *ent.* **6a**. The difference in activity toward seeds of *Striga hermonthica*, expressed as $c_{1/2max}$ (concentration at half-maximal activity) is more than 100, *cf.* entries 1 and 3, Table II. For seeds of *Orobanche crenata* this difference amounts to approximately a factor of 100 (fig. 4). The relative importance of the absolute configuration at the stereogenic centers C-3a,C-8b and C-2' on the bioactivity can be established by comparison of the activities of **6b** and *ent.* **6b** (entries 2 and 4, Table II; fig. 4). This reveals that these contributions are almost equal for both species, which is in contrast with the results obtained for the stereoisomers of GR7 (**5**), which indicate a more profound role of the configuration at C-2'.[17] Bergmann *et al.* concluded from the relative activities of four stereoisomers of strigol, that the absolute stereochemistry at C-2' is of special importance to exhibit maximal stimulatory activity.[19] However, it should be noted that the differences in

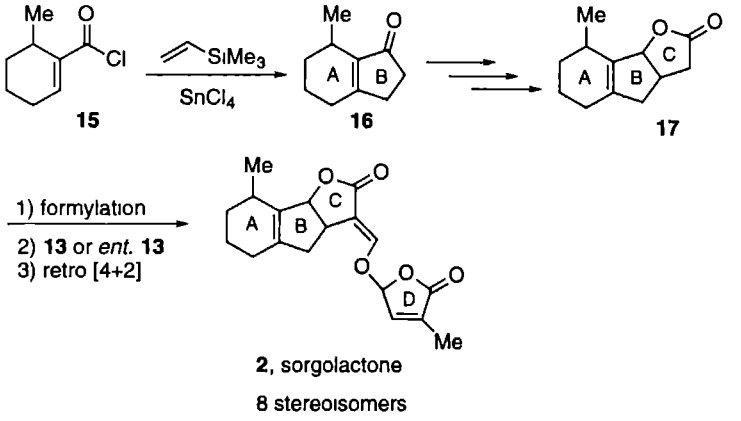
activity are only marginal. A similar comparative study for all stereoisomers of GR24 (**4**) revealed that the stereochemistry at C-3a,C-8b is of considerable importance for the stimulatory activity (*Orobanche crenata*) as compared to that of DMSL (**6**) (see chapter 5).[6]

Evaluation of all available data regarding the germination stimulatory activity of several optically pure analogues derived from (+)-strigol reveals that the absolute stereochemistry in the D-ring and BC-part are both essential to exert a maximal effect. Generalizations[17,19] suggesting a determining role of the correct configuration at C-2' are not allowed, as these data represent special cases and obviously not a general trend. The combination of the configurations at all stereogenic centers determines the molecular shape, and this will govern the interaction with the receptor.

6.3 Concluding remarks

A general route for the stereoselective synthesis of all stereoisomers of DMSL (**6**) has been achieved It was shown that the stereochemistry at all stereogenic centers has a considerable influence on the stimulatory activity. It should be noted that the synthetic sequence to DMSL (**6**) can, in principle, be followed for the asymmetric synthesis of sorgolactone (**2**) as is outlined in scheme 3.

Scheme 3



In a manner analogous to that used for **9**, bicyclic AB-fragment **16** can be prepared by a Nazarov-type cyclization of the pentadienyl cationic species that is generated upon the Lewis-acid mediated reaction of acid chloride **15** and trimethylvinylsilane, which is known in the literature [11] The transformation of **16** into sorgolactone (**2**) via the ABC-fragment **17** requires several similar synthetic operations as described above for the preparation of DMSL (**6**) and thus allows control of the absolute configuration at C-2'. Provided that the separation of diastereomers

is feasible at appropiate stages in the synthetic sequence, this route offers the opportunity to synthesize all eight single stereoisomers of sorgolactone.

6.4 Experimental section

Synthesis

General remarks

100 MHz ^{1}H-NMR and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 and Bruker AM-400 spectrometer, respectively (Me_4Si as internal standard). All coupling constants are given as ^{3}J in Hz, unless indicated otherwise. For mass spectra a double focussing VG7070E mass spectrometer was used. GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30m x 0.25 mm). Helium was used as carrier gas, and electron impact (EI) was used as ionization mode. GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were performed at the Department of Micro-analysis of this laboratory. CD-spectra were recorded using a Jasco J600 spectrophotometer.

Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH_2. Tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0.25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray. Flash-chromatography was carried out at a pressure of *ca.* 1.5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was carried out, using Merck Kieselgel 60.

Sodium hydride (60% in dispersion oil) was washed twice with hexane just before use. The syntheses of chloro lactones **13** and *ent.* **13**[7] and 5-bromo-3-methyl-2(5H)-furanone **8**[8] were reported previously. Bicyclic enone **9** was prepared following essentially the procedure according to Ramaiah.[11]

2-Ethoxycarbonylmethyl-3-oxo-2,3,4,5,6,7-hexahydro-1H-indene-2-carboxylic acid ethyl ester (**10**)

A solution of bicyclopentenone **9** (2.00 g, 14.7 mmol) in DMF (5 mL) was gradually added to a solution of diethyl carbonate (7.1 mL, 58.7 mmol) and sodium hydride (1.29 g, 32.3 mmol) in DMF (15 mL) with stirring at 65^{0}C. After stirring for 1h at 65^{0}C, a solution of ethyl bromoacetate (3.67 g, 22.0 mmol) in DMF (5 mL) was gradually added. After 2 h of stirring at the same temperature, the reaction mixture was neutralized with glacial acetic acid. The mixture was concentrated *in vacuo* and the residue was dissolved in a mixture of diethyl ether and water. The aqueous layer was extracted with diethyl ether (3x) and the combined organic layers were washed with water, dried ($MgSO_4$) and concentrated *in vacuo* to give crude **10**, which was sufficiently pure for further reactions. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 9:1) provided pure **10** (3.55 g, 82%) as a yellowish oil.

^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.15-1.31 (2t, 6H, J 7.1 Hz, 2 C$\underline{H}_3$), 1.71 (m, 4H, 2 =C-CH_2-C$\underline{H}_2$ A-ring), 2.16 (m, 2H, =C-C$\underline{H}_2$ A-ring), 2.37 (m, 2H, =C-C$\underline{H}_2$ A-ring), 2.45 and 3.29 (AB, 2H, ^{2}J

17.1 Hz, C$\underline{H}_2CO_2Et$), 2.54 and 3 24 (AB, 2H, ^2J 17 Hz, C$\underline{H}_2$ B-ring), 4.02-4 26 (m, 4H, 2 C$\underline{H}_2CH_3$) ppm; IR (CCl_4): ν 1750-1700 (several peaks, C=O), 1650 (C=C) cm^{-1}; MS (EI, m/z, rel int. (%)): 294 ([M]$^+$, 33), 249 ([$C_{14}H_{17}O_4$]$^+$, 46), 220 ([$C_{12}H_{12}O_4$]$^+$, 100), 148 ([$C_{10}H_{12}O$]$^+$, 46), 91 ([C_7H_7]$^+$, 21), HRMS/EI: m/z calcd for $C_{16}H_{22}O_5$: 294.1467. Found 294.14672 ± 0.00088.

3-Oxo-2,3,4,5,6,7-hexahydro-1H-inden-2-yl-acetic acid (**11**)
This compound was prepared as described for the synthesis of GR24 **4** (Mangnus *et al* , 1992b) Starting from diester **10** (4.55 g, 15.0 mmol) crude **11** (2.29 g, 80%, GC purity 99%) was obtained as a brownish oil. For characterization a small sample was triturated in di*iso*propyl ether to give **11** as a white solid
Mp 115-116^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.70 (m, 4H, 2 =C-CH_2-C$\underline{H}_2$ A-ring), 2 10-2 97 (m, 9H, 2 =C-C$\underline{H}_2$ A-ring, C$\underline{H}_2$ B-ring, C$\underline{H}$ B-ring, CH_2COOH), 11.0 (br s, 1H, COO$\underline{H}$) ppm, IR (KBr): ν 3300-2500 (broad, OH), 1735 (COOH), 1660 (C=O), 1630 (C=C) cm^{-1}; MS (CI, m/z, rel. int (%)). 294 ([M+1]$^+$, 100), 177 ([$C_{11}H_{13}O_2$]$^+$, 85), 149 ([$C_{10}H_{13}O_2$]$^+$, 69), 134 ([$C_9H_{10}O$]$^+$, 19), 91 ([C_7H_7]$^+$, 31).

3,3a,4,5,6,7,8,8b-Octahydro-indeno-[1,2-b]furan-2-one (**7**)
Procedure a: To a cooled (-78^{0}C) solution of **11** (806 mg, 4.15 mmol) in dichloromethane (25 mL) under nitrogen DIBALH (8.7 mL of a 1M solution in hexane) was gradually added using a syringe. After 5 min of stirring at -78^{0}C, the mixture was quenched with 20% H_2SO_4 (15 mL) and allowed to warm up to room temperature The aqueous phase was extracted with dichloromethane (3x). After drying ($MgSO_4$) the solvent was evaporated *in vacuo* and the residue was subjected to flash chromatography (SiO_2, hexane/ethyl acetate 4:1) to give **7** (472 mg, 64%) as a slightly brown oil, which solidified on standing. An analytical sample was obtained by crystallization from hexane/ethyl acetate to give **7** as white crystals
Mp 38 5-40^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.66 (m, 4H, $\underline{H}_6$ and $\underline{H}_7$), 2 04 (m, 4H, $\underline{H}_5$ and $\underline{H}_8$), 2 27 (dd, 1H, ^2J 17 6 Hz, J 5 1 Hz, $\underline{H}_3$), 2 84 (dd, 1H, ^2J 17.6 Hz, J 10.1 Hz, $\underline{H}_3$), 2.21-2 61 (m, 2H, $\underline{H}_4$), 3.09 (m, 1H, $\underline{H}_{3a}$), 5.30 (br d, 1H, J 7 0 Hz, $\underline{H}_{8b}$) ppm, IR (CCl_4): ν 1775 (C=O) cm^{-1}, HRMS/EI: m/z calcd for $C_{11}H_{14}O_2$: 178 0994. Found 178.09935 ± 0.00084.
Procedure b· Keto acid **11** (0.20 g, 1.0 mmol) was dissolved in 15 mL of a 0.13 M $CeCl_3$ $7H_2O$ methanol solution (2 eq.) and $NaBH_4$ (0.16 g, 4.0 mmol) was slowly added with stirring at room temperature. After 20 min. of stirring the reaction mixture was quenched with 20% H_2SO_4 (pH 1-2) and filtered over hyflo. Ethyl acetate (25 mL) was added to the filtrate and the organic phase was washed with satd. $NaHCO_3$ (3x), dried ($MgSO_4$) and concentrated *in vacuo* to give pure **7** (145 mg, 79%) as a slightly brown oil, which solified on standing. The analytical data were in complete agreement with those obtained in procedure a.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2-yloxymethylene)-3,3a,4,,5,6,7,8,8b-octahydro-indeno[1,2-b]furan-2-one (*rac.* **6a** *and rac* **6b**)
To a stirred suspension of NaH (46 mg, 1.90 mmol) in diethyl ether (10 mL) tricyclic lactone **7** (306 mg, 1.72 mmol) in diethyl ether (5 mL) was gradually added at room temperature under nitrogen. Ethyl formate (1.7 mL, 21 mmol) was added and stirring was continued for 15 h. The solvent was removed *in vacuo*. The thus obtained sodium salt **12** was dissolved in DMF (10 mL) A solution of bromofuranone **8** (333 mg, 1.87 mmol) in DMF (3 mL) was gradually added at -50^{0}C under nitrogen. After 17 h of stirring at room temperature the mixture was quenched with

acetic acid (0.5 mL) and the solvent was removed *in vacuo*. The residue was dissolved in a mixture of water and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2x) and the combined organic layers were washed with water (2x), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified using flash chromatography (SiO_2, hexane / ethyl acetate 2:1) to afford two diastereomeric products. Fast moving diastereomer *rac.* **6a** (166 mg, 32%) and slow moving diastereomer *rac.* **6b** (145 mg, 28%) were obtained as white solids. Analytical samples were obtained by recrystallization from 2-propanol.

rac. **6a**: Mp 148-150^{0}C; R_f 0.34 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.62 (m, 4H, $\underline{H}_6$, $\underline{H}_7$), 1.83-2.90 (m, 9H, 2 =C-C$\underline{H}_2$ A-ring, C$\underline{H}_3$, 2 $\underline{H}_4$), 3.52-3.75 (m, 1H, $\underline{H}_{3a}$), 5.32 (d, 1H, J 7.5 Hz, $\underline{H}_{8b}$), 6.16 (m, 1H, OC$\underline{H}$O D-ring), 6.93 (m, 1H, =C$\underline{H}$ D-ring), 7.43 (d, 1H, ^{4}J 2.5 Hz, =C$\underline{H}$O) ppm; MS (EI, m/z, rel. int. (%)): 302 ([M]$^+$, 0.2), 205 ([$C_{12}H_{13}O_3$]$^+$, 18.9), 97 ([$C_5H_5O_2$]$^+$, 100); Analysis calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.86; H, 5.94.

rac. **6b**: Mp 179-184^{0}C; R_f 0.24 (hexane/ ethyl acetate 1:1); ^{1}H-NMR and mass data were the same as for *rac.* **6a**; Analysis calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 66.72; H, 5.91.

3-(6(S)-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]dec-8-en-3(R)-yloxymethylene)-3,3a(R),4,5,6,7,8,8b(S)-octahydro-indeno[1,2-b]furan-2-one (**1 4 a**) *and its 3a(S),8b(R) -diastereomer* (*ent.* **14b**)

To a stirred suspension of NaH (68.0 mg, 1.70 mmol) in diethyl ether (10 mL) tricyclic lactone **7** (276 mg, 1.55 mmol) in diethyl ether (5 mL) was gradually added at room temperature under nitrogen. Ethyl formate (1.7 mL, 21 mmol) was added and stirring was continued for 15 h. The solvent was removed *in vacuo*. The thus obtained sodium salt **12** was dissolved in DMF (10 mL). A solution of chloro lactone **13** (308 mg, 1.55 mmol) in DMF (3 mL) was gradually added at room temperature under nitrogen. After 17 h of stirring the mixture was quenched with acetic acid (0.5 mL) and the solvent was removed *in vacuo*. The residue was dissolved in a mixture of water and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2x) and the combined organic layers were washed with water (2x), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified using flash chromatography (SiO_2, hexane / ethyl acetate 3:1) to afford two diastereomeric products. The fast moving diastereomer **14a** (203 mg, 36%) was obtained as a white solid, and crystallization from hexane/ ethyl acetate afforded analytically pure **14a**. The slow moving diastereomer *ent.* **14b** (261 mg, 46%) was obtained as a white solid, which gave an analytically pure sample after crystallization from hexane/ ethyl acetate.

14a: Mp 210-212^{0}C; $[\alpha]_D$ +169^0 (c 0.4, $CHCl_3$); R_f 0.51 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.58 (s, 3H, C$\underline{H}_3$), 1.62-1.72 (m, 6H, $\underline{H}_{10}$, 2 =C-CH$_2$-C$\underline{H}_2$ A-ring), 1.97-2.80 (m, 6H, 2 =C-C$\underline{H}_2$ A-ring, C$\underline{H}_2$ B-ring), 2.70 (dd, 1H, J 4.1 Hz, J <1 Hz, $\underline{H}_2$), 2.89 (m, 1H, $\underline{H}_7$), 3.23 (m, 1H, $\underline{H}_1$), 3.62 (m, 1H, $\underline{H}_{3a}$), 5.24 (d, 1H, J <1 Hz, $\underline{H}_3$), 5.29 (br d, 1H, J 7.2 Hz, $\underline{H}_{8b}$), 6.25 (m, 2H, $\underline{H}_8$ and $\underline{H}_9$), 7.35 (d, 1H, ^{4}J 2.5 Hz, =C$\underline{H}$O) ppm; MS (EI, m/z, rel. int. (%)): 368 ([M]$^+$, 1.7), 302 ([$C_{17}H_{18}O_5$]$^+$, 0.8), 206 ([$C_{12}H_{14}O_3$]$^+$, 13.2), 163 ([$C_{10}H_{11}O_2$]$^+$, 84.2), 97 ([$C_5H_5O_2$]$^+$, 100), 66 ([C_5H_6]$^+$, 13.0); Analysis calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.61; H, 6.58.

ent. **14b**: Mp 206-208.5^{0}C; $[\alpha]_D$ -227^0 (c 0.4, $CHCl_3$); R_f 0.37 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.57 (s, 3H, C$\underline{H}_3$), 1.62-1.72 (m, 6H, $\underline{H}_{10}$, 2 =C-CH$_2$-C$\underline{H}_2$ A-ring), 1.96-2.80 (m, 6H, 2 =C-C$\underline{H}_2$ A-ring, C$\underline{H}_2$ B-ring), 2.73 (dd, 1H, J 4.1 Hz, J <1 Hz, $\underline{H}_2$), 2.89 (m, 1H, $\underline{H}_7$), 3.22 (m, 1H, $\underline{H}_1$), 3.61 (m, 1H, $\underline{H}_{3a}$), 5.23 (d, 1H, J <1 Hz, $\underline{H}_3$), 5.28 (br d, 1H, J 7.0 Hz, $\underline{H}_{8b}$), 6.25 (m, 2H, $\underline{H}_8$ and $\underline{H}_9$), 7.33 (d, 1H, ^{4}J 2.6 Hz, =C$\underline{H}$O) ppm; Mass data were the same as for **14a**. Analysis calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.57; H, 6.54.

3-(6(R)-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]dec-8-en-3(S)-yloxymethylene)-3,3a(R),4,5,6,7,8,8b(S)-octahydro-indeno[1,2-b]furan-2-one (**14b**) *and its 3a(S),8b(R) diastereomer* (*ent.* **14a**)

These compounds were prepared in the same way as described for **14a** and *ent.* **14b**, starting from tricyclic lactone **7** (274 mg, 1.54 mmol) and chloro lactone *ent.* **13** (307 mg, 1.54 mmol). Yield 216 mg, 38% of fast moving diastereomer *ent.* **14a** as white solid and 135 mg, 24% of slow moving diastereomer **14b** as a white solid. Both compounds were crystallized from hexane/ethyl acetate to obtain analytically pure samples.

ent. **14a**: Mp 212.5-213^{0}C; $[\alpha]_D$ -178^0 (c 0.4, $CHCl_3$); Analysis calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.67; H, 6.46. ^{1}H-NMR and mass data were the same as for compound **14a**.

14b: Mp 208-209.5^{0}C; $[\alpha]_D$ +233^0 (c 0.4, $CHCl_3$); Analysis calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.65; H, 6.48. ^{1}H-NMR and mass data were the same as for compound *ent.* **14b**.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene)-3,3a(R),4,,5,6,7,8,8b(S)-octahydro-indeno[1,2-b]furan-2-one (**6a**)

Fast moving cycloadduct **14a** (169 mg, 0.46 mmol) was dissolved in *o*-dichlorobenzene (40 mL) and heated at 180^{0}C for 6 h. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, hexane/ ethyl acetate 3:1) to give **6a** (42 mg, 30%) as a solid. An analytical sample was obtained by crystallization from hexane/ ethyl acetate.

Mp 150.5-155^{0}C; $[\alpha]_D$ +281^0 (c 0.1, CH_2Cl_2); R_f 0.34 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.60-1.67 (m, 4H, H_6, H_7), 1.96-2.03 (m, 3H, =C-CH_2 A-ring), 2.03 (m, 3H, CH_3), 2.19 (m, 1H, =C-CH_2 A-ring), 2.34 (br d, 1H, ^{2}J 16.6 Hz, H_4), 2.73 (dd, 1H, ^{2}J 16.6 Hz, J 8.7 Hz, H_4), 3.64 (m, 1H, H_{3a}), 5.32 (d, 1H, J 7.5 Hz, H_{8b}), 6.15 (m, 1H, OCHO D-ring), 6.92 (m, 1H, =CH D-ring), 7.42 (d, 1H, ^{4}J 2.6 Hz, =CHO) ppm; Mass data were the same as for *rac.* **6a**; Analysis calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 66.88; H, 5.97.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(S)-yloxymethylene)-3,3a(S),4,5,6,7,8,8b(R)-octahydro-indeno[1,2-b]furan-2-one (*ent.* **6a**)

Prepared in the same way as described for **6a**, starting from *ent.* **14a** (250 mg, 0.68 mmol). Yield 73 mg, 36% of *ent.* **6a** as a white solid. An analytical sample was obtained by crystallization from hexane/ ethyl acetate.

Mp 153.5-155^{0}C; $[\alpha]_D$ -285^0 (c 0.1, CH_2Cl_2); Analysis calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.36; H, 6.00. ^{1}H-NMR and mass data were the same as for compound **6a**.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(S)-yloxymethylene)-3,3a(R),4,5,6,7,8,8b(S)-octahydro-indeno[1,2-b]furan-2-one (**6b**)

Prepared in the same way as described for **6a**, starting from **14b** (226 mg, 0.61 mmol). Yield 78 mg, 42% of **6b** as a white solid. An analytical sample was obtained by crystallization from hexane/ ethyl acetate.

Mp 179.5-183.5^{0}C; $[\alpha]_D$ +161^0 (c 0.4, CH_2Cl_2); R_f 0.24 (hexane/ ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.57-1.67 (m, 4H, H_6, H_7), 1.96-2.01 (m, 3H, =C-CH_2 A-ring), 2.03 (m, 3H, CH_3), 2.19 (m, 1H, =C-CH_2 A-ring), 2.33 (br d, 1H, ^{2}J 16.6 Hz, H_4), 2.70 (dd, 1H, ^{2}J 16.6 Hz, J 9.0 Hz, H_4), 3.62 (m, 1H, H_{3a}), 5.32 (d, 1H, J 7.7 Hz, H_{8b}), 6.13 (m, 1H, OCHO D-ring), 6.93 (m, 1H, =CH D-ring), 7.43 (d, 1H, ^{4}J 2.6 Hz, =CHO) ppm; Mass data were the same as for **6a**; Analysis calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.32; H, 6.00.

3-(4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene)-3,3a(S),4,5,6,7,8,8b(R)-octahydro-indeno[1,2-b]furan-2-one (*ent* **6b**)

Prepared in the same way as described for **6a**, starting from *ent* **14b** (170 mg, 0 46 mmol) Yield 43 mg, 31% of *ent* **6b** as a white solid An analytical sample was obtained by crystallization from hexane/ ethyl acetate

Mp 180-184^{0}C, $[\alpha]_D$ -150^0 (c 0 3, CH_2Cl_2), Analysis calcd for $C_{17}H_{18}O_5$ C, 67 54, H, 6 00 Found C, 67 14, H, 5 99 ^{1}H-NMR and mass data were the same as for compound **6a**

Biological activity

Seeds

Seeds of *Striga hermonthica* (Del) Benth (from *Sorghum bicolor* (L) Moench) and *Orobanche crenata* Forsk (from *Vicia faba* L) were harvested in Burkina Faso in 1994 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests Bioassays were carried out essentially following the procedure of Mangnus *et al* with minor modifications [21]

Preparation of test solutions

A compound to be tested was weighed out very accurately to the amount of 2 5 mg, dissolved in 5 mL of acetone p a and diluted with demineralized water to 25 mL Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0 1, 0 01 and 0 001 mg/L test compound and 0 2, 0 02, 0 002% and 0 0002% (v/v) acetone, respectively

Bioassays

For surface sterilization seeds of *Striga hermonthica* were subsequently exposed to 70% (v/v) ethanol for 5 min and sodium hypochlorite (2% active chlorine) for 2 min with agitation Seeds of *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation The seeds were then thoroughly rinsed with water and dried

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter, approximately 30-70 seeds per disk) in Petri dishes, each containing 2 disks (*Striga*) or 4 disks (*Orobanche*), wetted with water, and stored in the dark for 14 days at 20^{0}C for *Orobanche* seeds and at 30^{0}C for *Striga* seeds Then the conditioning water was removed and replaced by 100 µL of test solution per disk (*Orobanche*) or 3 mL per Petri dish (*Striga*) After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at the indicated temperatures, the germination percentage was determined under a microscope Seeds were considered to be germinated if the radical protruded through the seed coat

In each test series aqueous solutions with 0 2 % (v/v) acetone were used as negative control Test solutions of the stimulant GR24 (concentrations of 1, 0 1, 0 01 and 0 001 mg/L) were used as positive controls All tests were performed at least in duplicate, and in each test the germination percentages were determined on 6 disks (*Striga*) or 12 disks (*Orobanche*) per treatment

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Chapter 7*

Synthesis of a Phthaloyl Glycine derived Strigol Analog and its Germination Stimulatory Activity toward Seeds of the Parasitic Weeds *Striga hermonthica* and *Orobanche crenata*

Abstract: The newly designed strigol analog Nijmegen 1 (*rac.* **7**) was prepared in high overall yield starting from *N*-phthaloyl glycine. This relatively simple analog exhibits high bioactivity in the stimulation of germination of seeds of the parasitic weeds *Striga hermonthica* and *Orobanche crenata*. Nijmegen 1 was resolved in its enantiomers **7** and *ent.* **7** by using the homochiral latent D-rings **12** and *ent.* **12**. The enantiomers **7** and *ent.* **7** show significant differences in germination activity.

7.1 Introduction

An attractive control strategy for the eradication of *Striga* and *Orobanche* infested fields is the concept of suicidal germination, *i.e.* introduction of a germination stimulating agent into the soil prior to sowing in order to induce germination of the parasitic seeds in the absence of a host plant.[1] The first known naturally occurring germination stimulant, (+)-strigol **1**, was isolated from the root exudate of the false host cotton (*Gossypium hirsutum* L.).[2,3] Recently, (+)-strigol **1** was also identified in the root exudates of the *Striga* host plants maize (*Zea mays* L.) and proso millet (*Panicum miliaceum* L.).[4] In addition, some structurally closely related 'strigolactones'[5] have been identified in the root exudates of other *Striga* hosts, *viz.* sorgolactone **2**[6] and alectrol **3**.[7] However, strigolactones **1-3** are not suitable for weed control purposes, because their structures are too complicated to allow synthesis in an economically feasible manner. Therefore, several studies have been conducted aiming at synthetic analogs with a relative simple structure but with high germination stimulatory activity.[8-12] These studies mainly focus on the ABC-part of the strigolactones. In this part of the molecule a considerable structural variation is allowed to retain high biological activity. In contrast, modifications in the vinyl ether part or the D-ring lead to substantial loss of stimulatory activity.[13] It was concluded that the bioactiphore, *i.e.* the part of the molecule which is primarily responsible for the biological activity, resides in this part of the molecule.

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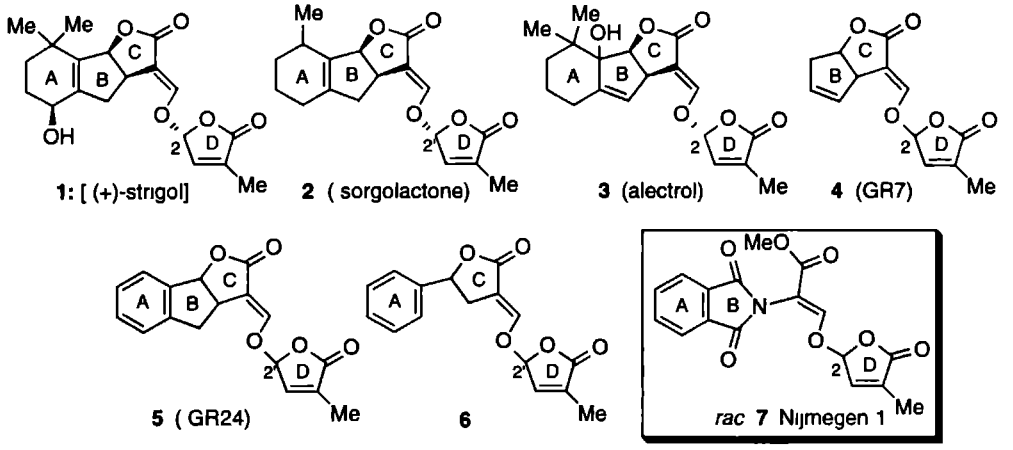


Figure 1

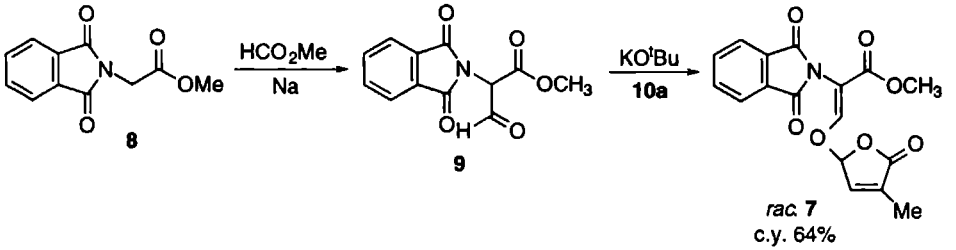
Important examples of highly potent synthetic analogs include **4** (GR7) and **5** (GR24) [9,14,15] Especially the latter, having an aromatic A-ring, is highly relevant, as its stimulatory activity is comparable to that of strigol[11] and its preparation is much easier than that of strigol [15] An even less complicated analog is compound **6** derived from commercially available γ-phenyl-γ-butyrolactone This analog, which lacks the B-ring, is almost as active as GR24 [12] A problem associated with the analogs **4-6** is the presence of two stereogenic centers in these molecules with the consequence that during their syntheses mixtures of diastereomers are obtained, which can be separated only by tedious chromatography This chapter deals with the synthesis of phthaloyl glycine derived strigol analog *rac* **7**, which we named Nijmegen 1, and the evaluation of the germination stimulatory activity toward seeds of *Striga* and *Orobanche* spp This new germination stimulant contains only one chiral center, namely in the D-ring In addition, the preparation of the individual enantiomers of the stimulant is described This newly designed strigol analog lacks the C-ring but still has the essential structural features, namely an enol ether contained in an enone unit and the butenolide D-ring

7.2 Results and discussion

Synthesis

The key step in the synthesis of *rac* **7** involves coupling of aldehyde **9** with 5-chloro-3-methyl-2(5H)-furanone **10a**, which was prepared according to scheme 2 Aldehyde **9** was synthesized by condensation of methyl *N*-phthaloylglycinate **8** with methyl formate using metallic sodium (scheme 1)

Scheme 1



This procedure, which closely resembles that described by Schutz,[16] is superior to that originally reported by Sheehan and Johnson.[17] It should be noted that **9**, commonly known as Sheehan aldehyde, is a stable crystalline compound, which can be stored for several years. The coupling reaction with butenolide **10a** (scheme 1) proceeds in high yield and purification was readily accomplished by recrystallization. It is important to note that only one geometrical isomer was obtained. The correct geometrical structure could not be deduced unambigiously by spectroscopic means and therefore an X-ray diffraction analysis was undertaken.[18] The structure of **7** is depicted in figure 2, showing that the Z-isomer was obtained.

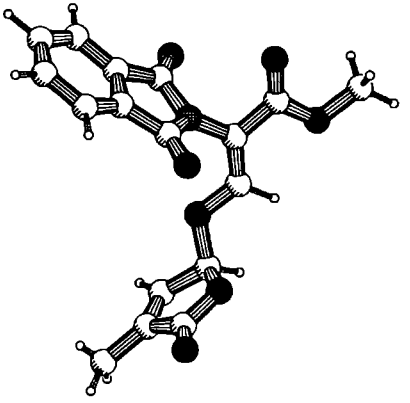
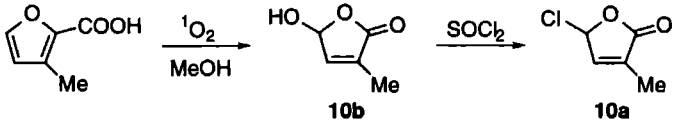


Figure 2. PLUTON generated drawing of X-ray crystal structure of Nijmegen 1 (*rac.* **7**)

The choice of chloro furanone **10a** as the electrophilic agent in the coupling reaction (scheme 1) deserves some comment. The yield obtained by using **10a** was higher than when the corresponding bromo furanone was employed. Moreover, chloro furanone **10a** is easier to handle as it is considerably more stable than its 5-bromo counterpart, and can be purified by distillation under reduced pressure without any problem. Its preparation is very convenient and involves chlorination using $SOCl_2$ of the corresponding hydroxy furanone **10b** (scheme 2).[9]

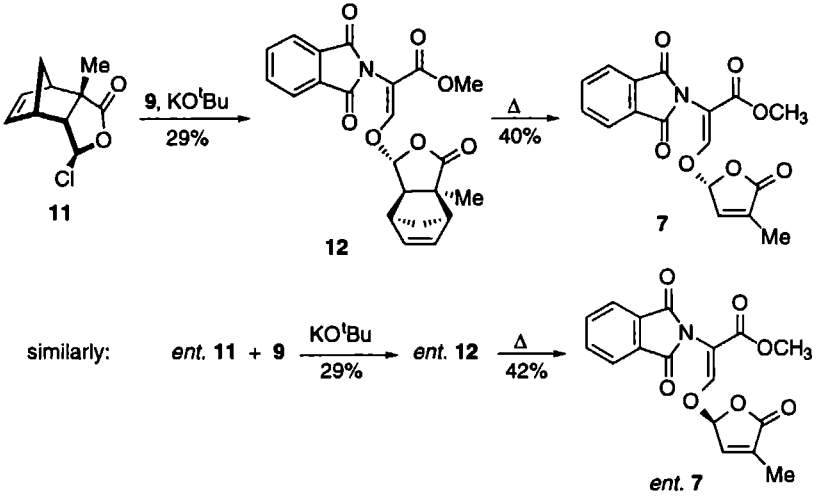
Scheme 2



Compound **10b** can best be prepared by photo-oxidation of readily available 3-methyl-2-furoic acid[19,20] with singlet oxygen, which is generated upon irradiation of a methanolic solution containing **10b** and oxygen in the presence of Rose Bengal as the sensitizer. This procedure, which is similar to that reported previously[21] allows the preparation of **10b** in a quantitative yield and can be performed on at least an one mole scale. These factors make the use of chloro furanone **10a** as the alkylating agent the method of choice in the coupling with precursors of strigol analogs (see chapter 8 for a convenient preparation of the corresponding bromo-furanone).

Next it was attempted to prepare the individual enantiomers of **7**, using enantiomerically pure tricyclic chloro lactones **11** and *ent.* **11** as the D-ring precursors (scheme 3). The stereoselective synthesis of **11** and *ent.* **11** and their use in the preparation of the single stereoisomers of strigol analog GR7 has been reported recently.[22]

Scheme 3



The coupling reactions of Sheehan aldehyde **9** with **11** and *ent.* **11**, respectively, did not proceed as smoothly as was observed for the corresponding GR7 analogs.[22] As a result of the relatively poor nucleophilicity of the enolate anion derived from **9**, a higher reaction temperature was required, which caused concomitant decomposition of **11** and *ent.* **11**. The result was not improved by the use of the corresponding *endo*-chloro epimers of **11** and *ent.* **11**. The cycloreversion of **12** and *ent.* **12** was performed in *o*-dichlorobenzene at 180^{0}C to give **7** and *ent.*

7, respectively, in moderate yields. The e.e.'s of both enantiomers were higher than 98%, as was determined by ^{1}H-NMR analysis using the chiral shift reagent Eu(hfc)$_3$.

Biological evaluation

The germination stimulatory activity of Nijmegen 1 (*rac.* **7**) was assayed using seeds of *Striga hermonthica* and *Orobanche crenata* spp. In each bioassay, GR24 was included as a positive control. The procedure enables a comparison between results obtained in different test series. This is important, since the response of seeds of parasitic weeds, especially *Striga hermonthica*, varies considerably from test to test. In addition, the activities of enantiomers **7** and *ent.* **7** were determined using seeds of *Orobanche crenata* spp. The results are collected in Tables I and II. It was beyond the aim of this study to establish complete dose-response curves, implying that the data obtained only allow an interpretation in a qualitative sense.

Table I. Germination percentages for seeds of *Striga hermonthica* after exposure to aqueous solutions of strigol analogs GR24 and Nijmegen 1 at concentrations of 1 mg/L and 0.01 mg/L[a]

		% germination ± S.E.	
entry	stimulant	1 mg/L	0.01 mg/L
1	GR24 (**5**)	41.2±4.6	45.0±5.4
2	Nijmegen 1(*rac.* **7**)	37.3±5.0	4.6±0.9
3	aqueous control[b]	5.6±1.0	5.5±1.1

[a] Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 550 seeds, distributed over 12 discs, of one representative experiment.

[b] Aqueous controls contain the same amount of acetone as the respective stimulant solutions

Table II. Germination percentages for seeds of *Orobanche crenata* after exposure to aqueous solutions of strigol analogs GR24 and Nijmegen 1 at concentrations of 2 mg/L, 1 mg/L, 0.1 mg/L and 0.01 mg/L[a]

			% germination ± S.E.		
entry	compound	2 mg/L	1 mg/L	0.1 mg/L	0.01 mg/L
1	GR24 (**5**)		66.1±1.8	46.5±1.0	4.5±0.7
2	Nijmegen 1(*rac.* **7**)	58.3±1.3	42.6±1.8	5.6±0.6	1.1±0.6
3	Nijmegen 1(**7**)		54.8±3.4	34.5±2.2	
4	Nijmegen 1(*ent.* **7**)		26.9±0.3	6.5±1.3	
5	aqueous control		0.2±0.2[b]		

[a] Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 500 seeds, distributed over 9 discs, of one representative experiment.

[b] The aqueous control contains the same amount of acetone as the respective stimulant solution

The data in Table I (*Striga hermonthica* spp.) reveal that *rac.* Nijmegen 1 exhibits considerable activity at the higher concentration of 1 ppm, whereas it is practically inactive at a concentration of 0.01 ppm. Similarly, *rac.* **7** gives only at higher concentrations comparable germination percentages of *Orobanche crenata* seeds as GR24 (entries 1 and 2, Table II). Comparison of the germination percentages exerted by enantiopure **7** and *ent.* **7** (entries 3 and 4, Table II), reveals that the former is considerably more active. The germination percentage found for **7** at a concentration of 0.1 ppm is high as compared to that of *rac.* **7**. Apparently, small differences in the concentration (factor 2) exhibit large differences in the response at this concentration interval.

Thus, the absolute stereochemistry at C-2' in the D-ring should be the *R*-configuration to germinate a maximum number of seeds. This configuration is the same as in natural (+)-strigol. This result is in agreement with previous conclusions from comparitive studies of the bioactivity of all stereoisomers of GR7[14], GR24 (chapter 5), DMSL (chapter 6) and of some stereoisomers of strigol,[11] namely that the most active stereoisomer has the *R*-configuration at C-2' in the D-ring.

7.3 Concluding remarks

From the results presented above, it may be concluded that phthaloyl glycine derived strigol analog *rac.* **7** is a potent germination stimulant of seeds of *Striga hermonthica* and *Orobanche crenata* spp. The charm of this particular stimulant is the fact that its racemic preparation is very simple and that it can be carried out without any chromatographic separation, which makes it an attractive compound for large-scale preparations and accordingly for use in the suicidal approach in the weed pest control. Moreover, the achiral ABC-part in *rac.* **7** enables a rapid evaluation of the influence of structural variations in the D-ring on the stimulatory activity, results of which are presented in chapter 9.

7.4 Experimental section

Synthesis

General remarks

100 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard) and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me_4Si as internal standard). All coupling constants are given as ^3J in Hz, unless indicated otherwise. For mass spectra a double focussing VG7070E mass spectrometer was used. GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.

Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH_2. Tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical

grade. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0.25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray. Flash-chromatography was carried out at a pressure of *ca.* 1 5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was carried out, using Merck Kieselgel 60.
The synthesis of chloro lactones **11** and *ent.* **11** has been reported previously.[22]

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-oxo-propionate (**9**)
To a cooled (-10^{0}C) solution of **8** (65.8 g, 300 mmol) in methyl formate (400 mL) small pieces of sodium (6.90 g, 300 mmol) were gradually added, with mechanical stirring in a nitrogen atmosphere . Stirring was continued for 18 h until all sodium had dissolved The reaction mixture was concentrated *in vacuo* and to the residue was added a mixture of glacial acetic acid (25 mL) and 1N HCl (50 mL). Crude **9** was obtained by extraction with dichloromethane (3x), drying ($MgSO_4$) and concentration *in vacuo*. Recrystallization from toluene gave pure **9** (59.3 g, 80%) as a pale yellow powder, with physical properties, identical with those reported previously.[17]

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxy)-acrylate (*rac* **7**)
Potassium *tert*-butoxide (372 mg, 3 32 mmol) was added to a cooled (0^{0}C) and stirred solution of Sheehan aldehyde **9** (745 mg, 3.02 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chloro furanone **10a** (480 mg, 3.62 mmol) in DMF (3 mL) was gradually added. The mixture was stirred at room temperature over weekend. DMF was removed *in vacuo* and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were washed with water (2x), dried ($MgSO_4$) and concentrated *in vacuo*. The oily residue was triturated with di*iso*propyl ether. Almost pure *rac.***7** (660 mg, 64%) was isolated as a white solid by filtration and washing with di*iso*propyl ether. An analytical sample was obtained by recrystallization from 2-propanol Mp 151-152^{0}C, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.97 (br s, 3H, C$\underline{H}_3$), 3.78 (s, 3H, OC$\underline{H}_3$), 6.17 (br s, 1H, OC$\underline{H}$O), 6.90 (br s, 1H, =C$\underline{H}$), 7.76 (m, 2H, 2 arom. $\underline{H}$), 7 90 (m, 3H, 2 arom. $\underline{H}$ + =C$\underline{H}$O) ppm; MS (EI, m/z, rel. int. (%))· 343 ([M]$^+$, 2.7), 246 ([$C_{12}H_8NO_5$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 59.3); Analysis calcd for $C_{17}H_{13}NO_7$: C, 59.48; H, 3.82; N, 4 08 Found: C, 59.10; H, 3.85; N, 4.00.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(6(S)-methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]-dec-8-en-3(R)-yloxy)-acrylate (**12**)
Potassium *tert*-butoxide (149 mg, 1.33 mmol) was added to a stirred solution of Sheehan aldehyde **9** (302 mg, 1 22 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chloro lactone **11** (265 mg, 1.33 mmol) in DMF (3 mL) was gradually added The mixture was stirred at 55^{0}C for 7 days and then quenched with acetic acid (0 5 mL). DMF was removed *in vacuo* and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (2x) The combined organic layers were washed with water (2x), dried ($MgSO_4$) and concentrated *in vacuo* The crude product was purified by flash chromatography (SiO_2, hexane / ethyl acetate 2:1) to give **12** (143 mg, 29%) as a yellowish solid. An analytical sample was obtained by recrystallization from di*iso*propyl ether/ ethyl acetate.

Mp 179.5-181^{0}C; $[\alpha]_D$ -22^0 (c 0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.46 (s, 3H, $C\underline{H}_3$), 1.65 (m, 2H, $\underline{H}_{10}$), 2.62 (dd, 1H, J 4.2 Hz, J <1 Hz, $\underline{H}_2$), 2.85 (m, 1H, $\underline{H}_7$), 3.16 (m, 1H, $\underline{H}_1$), 3.77 (s, 3H, $OC\underline{H}_3$), 5.26 (d, 1H, J <1 Hz, $\underline{H}_3$), 6.17 (m, 1H, $\underline{H}_9$), 6.28 (m, 1H, $\underline{H}_8$), 7.77 (m, 2H, Ar $\underline{H}$), 7.85 (s, 1H, =C$\underline{H}$O), 7.91 (m, 2H, Ar $\underline{H}$) ppm; MS (EI, m/z, rel. int. (%)): 409 ([M]$^+$, 0.6), 344 ([$C_{17}H_{14}NO_7$], 0.5), 247 ([$C_{12}H_9NO_5$], 43.6), 163 ([$C_{10}H_{11}O_2$], 78.2), 97 ([$C_5H_5O_2$], 100), 66 ([C_5H_6], 13.5); Analysis calcd for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.43. Found: C, 64.52; H, 4.63; N, 3.48.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(6(R)-methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]-dec-8-en-3(S)-yloxy)-acrylate (*ent.* **12**)

This compound was prepared in the same way as described for **12**, starting from Sheehan aldehyde **9** (601 mg, 2.43 mmol) and chloro lactone *ent.* **11** (530 mg, 2.67 mmol). Yield 286 mg, 29% of *ent.* **12** as a yellowish solid. Recrystallization from di*iso*propyl ether/ ethyl acetate afforded analytically pure *ent.* **12**.

Mp 179.5-181^{0}C; $[\alpha]_D$ +23^0 (c 0.2, $CHCl_3$); Analysis calcd for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.43. Found: C, 64.50; H, 4.64; N, 3.47. ^{1}H-NMR and mass data were the same as for compound **12**.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxy)-acrylate (**7**)

Cycloadduct **12** (159 mg, 0.39 mmol) was dissolved in *o*-dichlorobenzene (40 mL) and heated at 180^{0}C for 7 h. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, hexane/ ethyl acetate 1:1) to give **7** (53 mg, 40%) as a colorless oil, which failed to crystallize.

$[\alpha]_D$ +124^0 (c 0.15, CH_2Cl_2); ^{1}H-NMR and mass data were the same as for compound *rac.* **7**.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydro-furan-2(S)-yloxy)-acrylate (*ent.* **7**)

Prepared in the same way as described for **7**, starting from *ent.* **12** (230 mg, 0.56 mmol). Yield 43 mg, 31% of *ent.* **7** as a colorless oil, which failed to crystallize.

$[\alpha]_D$ -128^0 (c 0.15, CH_2Cl_2); ^{1}H-NMR and mass data were the same as for compound **7**.

Biological activity

Seeds

Seeds of *Striga hermonthica* (Del.) Benth (from *Sorghum bicolor* (L.) Moench) and *Orobanche crenata* Forsk. (from *Vicia faba* L.) were harvested in Sudan in 1988 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of test solutions

A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a. and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 2, 1, 0.1, and 0.01 mg/L test compound and 0.2, 0.1, 0.01, and 0.001% (v/v) acetone, respectively.

Bioassays

For surface sterilization seeds of *Striga hermonthica* and *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30-70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20°C for *Orobanche* seeds and at 30°C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.1, 0.01 and 0.001% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (concentrations of 1, 0.1 and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks (*Striga*) or 9 disks (*Orobanche*) per treatment.

For full details of the bioassay, see ref. 23.

7.5 References

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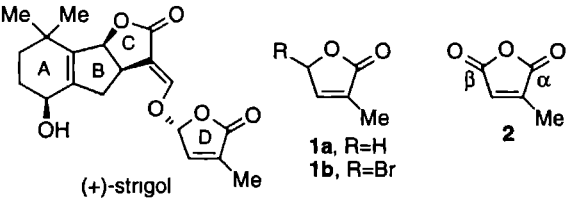
Chapter 8*

A Novel and Convenient Synthesis of 3-Methyl-2(5H)-Furanone

Abstract: 3-Methyl-2(5H)-furanone **1a**, a precursor of strigol and its analogues, is prepared in a highly efficient manner by a regiocontrolled alcoholysis of citraconic anhydride and subsequent reduction *via* the mixed anhydride **5c**.

8.1 Introduction

The 3-methyl-2(5H)-furanone moiety **1a** is a common structural feature of all known 'strigolactones', such as (+)-strigol. These compounds are naturally occurring germination stimulants of seeds of the parasitic weeds *Striga* and *Orobanche* spp.[1-4] Moreover, structure-activity relationship studies revealed that the presence of this structural unit is essential to retain full biological activity, results of which are reported in chapter 9.



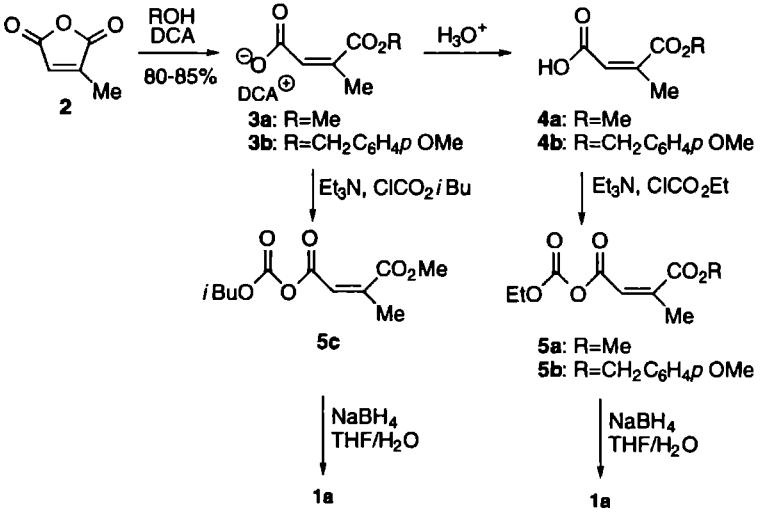
In view of our interest in the synthesis of simple, biologically active analogues of strigol, which are suitable for weed control purposes,[5,6] a convenient multi-gram preparation of **1a** is required, using cheap chemicals. This compound can readily be transformed into the corresponding 5-bromo derivative **1b**, which is a D-ring precursor in the synthesis of the strigolactones and their analogues.[7] Several procedures for the synthesis of **1a** have been reported, but none of them fulfils these criteria satisfactorily.[6,8-13] This chapter deals with an improved procedure for the preparation of 3-methyl-2(5H)-furanone **1a**

*This chapter has been accepted for publication in *Synthesis* by Nefkens, G H L , Thuring, J W J F , Zwanenburg, B

8.2 Results and discussion

An attractive cheap, commercially available starting material is citraconic anhydride **2**, as it only requires a formal reduction of the β-carbonyl function. However, this approach is not feasible as such, because reduction of the sterically most hindered α-carbonyl function strongly prevails. This observation was supported by *ab initio* calculations, showing a larger LUMO coefficient on the α-carbonyl.[14] This implies that nucleophilic attack takes place preferentially at the α-carbonyl, which is thus primary determined by electronic factors. The intrinsic difference in reactivity of both carbonyl functions of **2** could advantageously be used to accomplish the reduction in the desired regiocontrolled fashion in an indirect manner, as is outlined in scheme 1.

Scheme 1.



Alcoholysis of **2** in the presence of dicyclohexylamine (DCA) with either methanol or *p*-methoxybenzyl alcohol gave the esters **3a** and **3b**, respectively, isolated as the DCA-salts, in high yield (80%) and with high regioselectivity (>90%). In the first approach the DCA-salts **3a,b** were converted into the corresponding carboxylic acids **4a,b** by acidification with citric acid or potassium hydrogensulphate, followed by treatment with ethyl chloroformate in the presence of triethylamine to give the mixed anhydrides **5a,b**. Removal of the $Et_3N.HCl$ precipitate by filtration, immediately followed by addition of the filtrate containing **5a,b**, to a saturated aqueous solution of sodium borohydride, smoothly produced **1a**.[15] After conventional work-up, butenolide **1a** was isolated in a high overall yield (~80% from **3a,b**) after purification by fractional distillation under reduced pressure. The choice of the *p*-methoxybenzyl ester was advantageous because carboxylic acid **4b** is much more stable than **4a**. However, the formation of *p*-methoxybenzyl alcohol during the reduction process severely complicated the purification of **1a**

by distillation. A considerable improvement of the above procedure is the direct formation of mixed anhydride **5c** from **3a** (scheme 1). This could be accomplished by treatment of **3a** with *iso*butyl chloroformate, which circumvented the need of isolating carboxylic acid **4a**. In this experimental set-up ethyl chloroformate is not a suitable reagent, as a considerable amount of the corresponding ethyl ester of **4a** was formed under these conditions. The mixed anhydride **5c** was then immediately subjected to reduction with $NaBH_4$, using a reversed addition procedure, *i.e.* addition of a saturated aqueous solution of $NaBH_4$ to **5c**, which avoids a laborious extractive work-up. Crude butenolide **1a** contained a small amount (*ca.* 1%) of two by-products, *viz* 3-methyl-2,3-dihydro-2(3H)-furanone and an as yet unidentified polar product. It is essential to remove this polar by-product as it substantially suppressed the radical bromination reaction to give **1b** (*vide supra*). This can be achieved by a quick filtration over silica gel. Pure butenolide **1a** was thus obtained in a high overall yield (>80% from **3a**) after fractional distillation.

In conclusion, a convenient and simple preparation of 3-methyl-2(5H)-furanone **1a**, starting from citraconic anhydride **2**, has been accomplished by making use of the intrinsic difference in reactivity of both carbonyl groups in citraconic anhydride **2**. The procedure can readily be performed on at least a 0.5 mole scale using cheap ingredients and standard laboratory equipment. Alternative convenient procedures involve the use of α-methylene-γ-butyrolactone[13] or α-methyl-γ-butyrolactone[12], which are very expensive starting materials or otherwise difficult to obtain. The corresponding chloro furanone (**1**, R=Cl) can best be prepared in five steps using the hydroxy furanone (**1**, R=OH) as the precursor, as is outlined in chapter 7. This newly developed method is therefore superior to all previously reported syntheses.

8.3 Experimental section

General remarks
100 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard). All coupling constants are given as ^3J in Hz, unless indicated otherwise. GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.
Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Ethyl acetate was distilled from K_2CO_3. Tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade.

DCA-salt of 2-methyl-but-2-enedioic acid 1-methyl ester (**3a**)
To a cooled (-15^{0}C) solution of citraconic anhydride **2** (56 g, 0.5 mol) in methanol (400 mL) dicyclohexylamine (1.1 eq) was gradually added. The reaction mixture was stirred for 30 min at room temperature and then concentrated *in vacuo*, while keeping the temperature below 25^{0}C. Ethyl acetate was added to the residue and after 1h the product was isolated by filtration and washed with ethyl acetate to give **3a** as a white solid (138 g, 85%). An analytical sample was obtained after recrystallization from 2-propanol. Mp 121-122^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ

1.0-2.1 (m, 20H, cyclohexyl), 1.93 (d, 3H, ^{4}J 1.5 Hz, =CC$\underline{H}_3$), 2.8-3.2 (m, 2H, C$\underline{H}$N), 3.70 (s, 3H, OC$\underline{H}_3$), 6.05 (q, 1H, ^{4}J 1.5 Hz, =C$\underline{H}$), 9.63 (br s, 2H, N$\underline{H}_2$) ppm; Analysis calcd for $C_{18}H_{31}NO_4$: C, 66.43; H, 9.6; N, 4.3. Found: C, 66.17; H, 9.56; N, 4.41.

DCA-salt of 2-methyl-but-2-enedioic acid 1-(4-methoxy-benzyl) ester (**3b**)
To a stirred solution of citraconic anhydride **2** (5.6 g, 0.05 mol) in ethyl acetate (50 mL) *p*-methoxybenzyl alcohol (8.3 g, 0.06 mol) was added. The solution was cooled (-20^{0}C), followed by slow addition of dicyclohexylamine (10.0 g, 0.055 mol), which resulted in the formation of a white precipitate of **3b**. Stirring was continued for 1h at room temperature. The product was isolated by filtration, washed with ethyl acetate, to give **3b** as a white solid (17.5 g, 80%). An analytical sample was obtained after recrystallization from 2-propanol. Mp 114-115^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.0-2.1 (m, 20H, cyclohexyl), 1.92 (d, 3H, ^{4}J 1.5 Hz, =CC$\underline{H}_3$), 2.8-3.2 (m, 2H, C$\underline{H}$N), 3.79 (s, 3H, OC$\underline{H}_3$), 5.10 (s, 2H, C$\underline{H}_2$), 6.02 (q, 1H, ^{4}J 1.5 Hz, =C$\underline{H}$), 6.85 (m, 2H, arom. $\underline{H}$), 7.30 (m, 2H, arom. $\underline{H}$), 9.63 (br s, 2H, N$\underline{H}_2$) ppm; Analysis calcd for $C_{25}H_{37}NO_5$: C, 69.58; H, 8.64; N, 3.24. Found: C, 69.11; H, 8.67; N, 3.36.

2-Methyl-but-2-enedioic acid 1-(4-methoxy-benzyl) ester (**4b**)
A suspension of DCA-salt **3b** (2.0 g, 4.6 mmol) in a mixture of water (10 mL) and ethyl acetate (25 mL) was acidified by adding potassium bisulphate until pH<3, which resulted in a clear two-phase system. The aqueous phase was separated and the organic layer was dried ($MgSO_4$), concentrated *in vacuo* and crystallized from 2-propanol to give **4b** as a white solid in 80% yield. Mp 75-76^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.08 (d, 3H, ^{4}J 1.6 Hz, =CC$\underline{H}_3$), 3.79 (s, 3H, OC$\underline{H}_3$), 5.17 (s, 2H, C$\underline{H}_2$), 5.86 (q, 1H, ^{4}J 1.6 Hz, =C$\underline{H}$), 6.87 (m, 2H, arom. $\underline{H}$), 7.27 (m, 2H, arom. $\underline{H}$) ppm; Analysis calcd for $C_{13}H_{14}O_5$: C, 62.39; H, 5.63. Found: C, 62.14; H, 5.84.

3-Methyl-2(5H)-furanone (**1a**)
To a cooled (-10^{0}C) solution of DCA-salt **3a** (65 g, 0.20 mol) in dichloromethane (150 mL) *iso*butyl chloroformate (30 g, 0.22 mol) was gradually added with stirring. During the addition a precipitate of dicyclohexylamine chlorohydrate gradually settled. The mixture was stored overnight at ca. -10^{0}C. Then THF (150 mL) was added and the mixture was allowed to stand for 1h at the same temperature. The precipitate was removed by filtration, while cooling the filtrate (0^0), and washed with THF (150 mL). To the filtrate containing mixed anhydride **5c**, a cold solution of sodium borohydride (15 g, 0.4 mol) in water (30 mL) was added at 0^{0}C, while stirring vigorously, over an 1h period. Stirring was continued for 2h at room temperature and the precipitate was removed by filtration and washed with diethyl ether. The filtrate was carefully concentrated *in vacuo* and the residue was dissolved in di*iso*propyl ether and dried ($MgSO_4$). The solvent was removed *in vacuo* to give **1a** as a colorless oil, which was purified by fractional distillation at low pressure and subsequently passed over a short column of silica gel, using carbon tetrachloride as the eluent. Yield 17 g (80%) as a colorless oil; bp 80^{0}C (15 Torr). The ^{1}H-NMR data were in full agreement with those reported.[16]

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Chapter 9*

N-Phthaloyl Glycine Derived Strigol Analogues. Influence of the D-ring on Seed Germination Activity of the Parasitic Weeds *Striga hermonthica* and *Orobanche crenata*

Abstract: Several strigol analogs with modifications in the D-ring were synthesized and assayed for germination stimulatory activity of seeds of *Striga hermonthica* and *Orobanche crenata* All these D-ring analogs are derived from *N*-phthaloyl glycine as the common ABC-fragment It was concluded that the structure of the 2(5H)-furanone D-ring is essential to retain full biological activity

9.1 Introduction

The strigolactones,[1] *viz* (+)-strigol **1**,[2-4] sorgolactone **2**[5] and alectrol **3**[6] are highly active germination stimulants of seeds of the parasitic weeds *Striga* and *Orobanche*

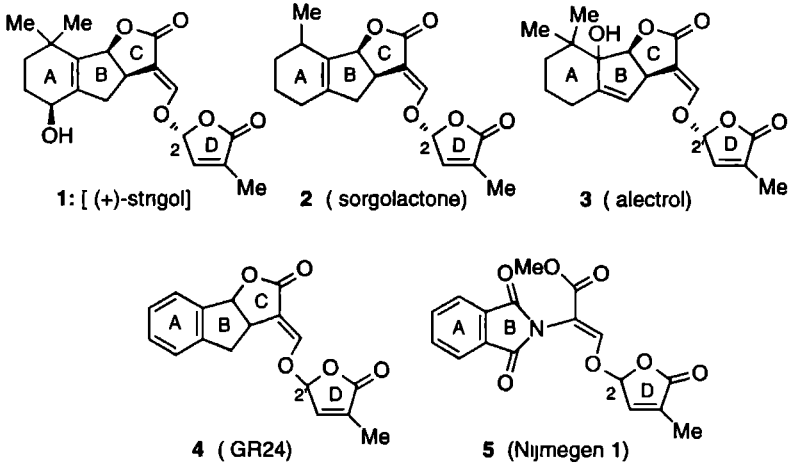

Figure 1

These compounds have been identified in the root exudates of some *Striga* hosts They may, in principle, find applications for the control of these parasitic weeds, because they are essential

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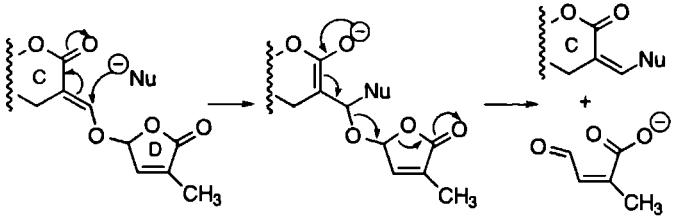
regulatory factors in the germination of the weed seeds. However, their complex structures and poor stabilities are severe limitations to be applicable as such. Therefore, several studies studies have been conducted to design synthetic analogs with the aim to obtain relatively simple compounds possessing high germination stimulatory activity, and to locate the bioactiphore, *i.e.* the part of the molecule, which is primary responsible for the biological activity.[7-11] These studies have mainly been focussed on the ABC-part of the strigolactones. It was concluded that there exists a relatively large degree of structural freedom in this part of the molecule to retain full biological activity. One of the most potent synthetic strigol analogs is GR24 (**4**), containing an aromatic A-ring,[7,8] the preparation of which is much easier than that of strigol **1**.[12] Further structure-bioactivity relationship studies revealed that the connecting enol ether unit is essential for stimulatory activity. This finding has led to the proposal of a molecular mechanism for germination (scheme 1) in which the bioactiphoric CD-part plays a key role.[13]

Scheme 1



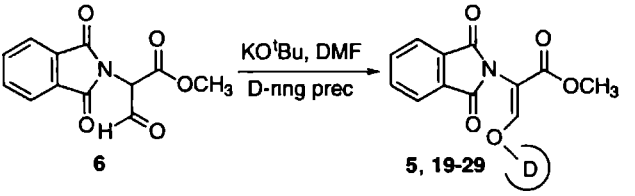
Thus far, relatively little attention has been paid to the influence of the structure of the D-ring on the activity. It was demonstrated that replacement of this 2(5H)-furanone moiety by other substituents leads to complete loss of bioactivity.[10,13]

This chapter deals with the influence of the structure of the D-ring on the germination stimulatory activity of *Striga hermonthica* (Del.) Benth. and *Orobanche crenata* Forsk. seeds. In order to make such an evaluation feasible, several five-membered ring analogs of the 3-methyl-2(5H)-furanone moiety, as is present in the strigolactones **1-3** and GR24 (**4**), were synthesized and subsequently coupled with a common ABC-fragment. Strigol analog Nijmegen 1 (**5**), derived from *N*-phthalimido glycine, was selected as the reference compound, the preparation of which has been reported recently in chapter 7. It was demonstrated that **5** exhibits considerable activity as a germination stimulant. An important benefit of using **5** as the reference compound is that it contains an achiral ABC-fragment, which precludes the formation of a diastereomeric mixture after its coupling with a D-ring precursor. Moreover, the preparation of **5** is simple, because readily available Sheehan aldehyde **6** (scheme 1) is used as the ABC-precursor.

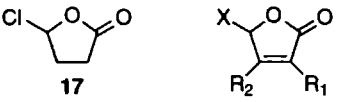
9.2 Results and discussion

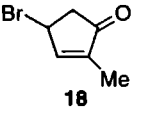
Synthesis

Scheme 2



D-ring precursors:





7 R_1=Me, R_2=H, X=Br (**7a** X=H)
8 - R_1- R_2 - = - CH=CH-CH=CH-, X=Br (**8a** X=H)
9 R_1=H, R_2=H, X=Br (**9a** X=H)
10 R_1=H, R_2=Me, X=Br (**10a** X=H)
11 R_1=Me R_2=Me, X=Cl (**11a** X=OH)
12 R_1=Me, R_2=OMe X=Br (**12a** X=H)
13 R_1=Et, R_2=H, X=Br (**13a** X=H)
14 R_1=Ph, R_2=H, X=Br (**14a** X=H)
15 R_1=H, R_2=Ph, X=Br (**15a** X=H)
16 R_1=Cl R_2=Cl X=OH[a]

a Coupling conditions DCC/DMAP in THF

Table 1. Yields of coupling reactions of **6** and D-ring precursors **7-18**

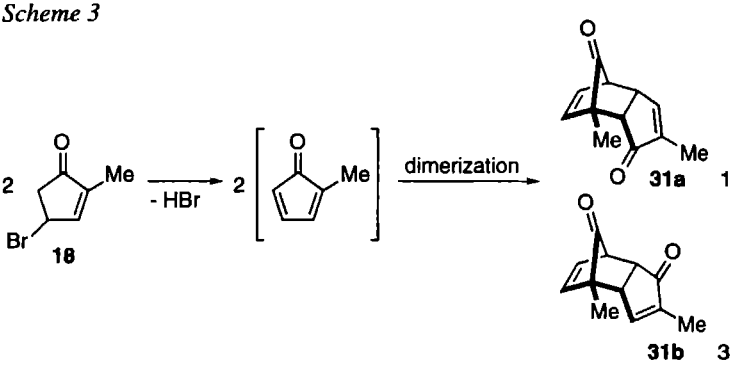
entry	D-ring precursor	product	yield (%)
1	**7**	**5**	75
2	**8**	**19**	55[a]
3	**9**	**20**	37
4	**10**	**21**	12[a]
5	**11**	**22**	95
6	**12**	**23**	85
7	**13**	**24**	b
8	**14**	**25**	21
9	**15**	**26**	27
10	**16**	**27**	35
11	**17**	**28**	49[a]
12	**18**	**29**	15

[a] Yield after purification by recrystallization
[b] not determined, see text

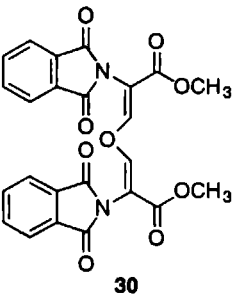
The final step in the synthesis of D-ring analogs **5** and **19-29** involves coupling (O-alkylation) of Sheehan aldehyde **6** (chapter 7) with D-rings **7-18** (scheme 2). These coupling reactions involve nucleophilic substitution of the corresponding γ-bromo or γ-chloro derivatives **7-15** and **17-18**, according to the general procedure for the preparation of strigol and its analogs,[8] using DMF as the solvent. This procedure provided the desired products in moderate to high yields (Table 1).

The D-ring precursors **7-15** and **17-18** were freshly prepared before use, because they are generally rather unstable. The poor stability of these compounds may account for some of the low yields of the desired products (Table 1), *e.g.* during several attempts to prepare carba D-ring analog **29**. Under the alkaline reaction conditions rapid dehydrobromination occurred, followed by dimerization of the highly reactive cyclopentadienone intermediate to give tricyclic pentenones **31a,b** in a ratio 3:1 (scheme 3).

Scheme 3



The estimated yield of **31a,b** was 60% based on ^{1}H-NMR analysis of the crude reaction mixture. Dehydrobromination and subsequent spontaneous dimerization of cyclopentadienones is known to occur rapidly.[14-16] Bis chloro-substituted analog **27** was prepared in an alternative manner, employing DCC-mediated coupling conditions in THF with commercially available mucochloric acid **16**. It was essential to carry out the reaction in the presence of a catalytic amount of DMAP, which enhanced the solubility of **16** considerably. In the absence of DMAP, predominantly dimerization of **6** occurred to give **30**, which was isolated in a yield of 63%.



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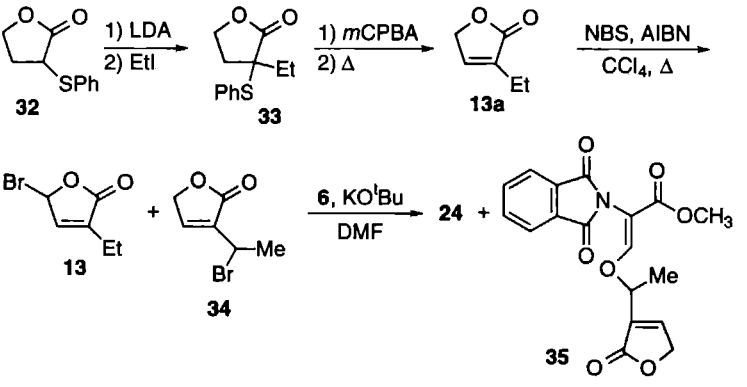
Preparation of D-ring precursors 7-18

All 5-bromo-2(5H)-furanones were prepared by radical initiated allylic bromination of the parent 2(5H)-furanones.[17] 5-Chloro-3,4-dimethyl-2(5H)-furanone **11** was prepared in three steps starting from propionaldehyde and ethyl pyruvate.[18,19] γ-Chloro-γ-butyrolactone **17** was obtained in one step by reaction of succinyl dichloride and tri-*n*-butyltin hydride.[20] 4-Bromo-2-methyl-2-cyclopenten-1-one **18** was obtained by bromination of 2-methyl-2-cyclopenten-1-one[21] with NBS.[16] The preparation of 4-methoxy-3-methyl-2(5H)-furanone **12a** was accomplished by methylation of the parent 2-methyl tetronic acid with dimethyl sulfate.[22] The last-mentioned compound was prepared in a one-pot synthesis by bromination of ethyl 2-methyl-acetoacetate to give the 2-bromo derivative,[23] which rearranged in the presence of hydrobromic acid to the 4-bromo isomer, that on heating gave 2-methyl tetronic acid.[24] The synthesis of 3-ethyl-2(5H)-furanone **13a** was carried out, using the concept as is outlined in scheme 4.

Scheme 4

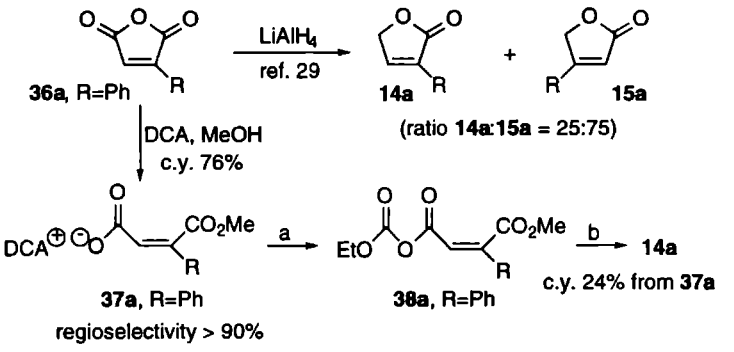


The sequence of α-alkylation using methyl iodide[25] or α-condensation with simple aldehydes[26] of phenylsulfanyl-γ-butyrolactone **32**, followed by oxidation and thermal dehydrosulfenylation, is a well-established route to 3-substituted 2(5H)-furanones. The scope, however, is rather limited. In our hands, quenching the lithio enolate of **32** with ethyl iodide gave **33** in 60% isolated yield, which upon oxidation with *m*CPBA and thermal elimination afforded **13a** in 40% yield after distillation. Compound **13a** has previously been prepared by other methods, although no analytical data were reported.[27,28] Subsequent bromination with NBS in the presence of α,α'-azodi*iso*butyronitrile (AIBN) gave a mixture of mainly two monobrominated furanones **13** and **34** in an equimolar ratio (scheme 4). Regioisomers **13** and **34** could not be separated. Therefore, coupling with Sheehan aldehyde **6** was achieved using the crude mixture of **13** and **34** to provide **24** and **35** in 16% and 23% isolated yields, respectively.

The syntheses of 3- and 4-phenyl substituted 2(5H)-furanones **14a** and **15a** were accomplished via reduction of maleic anhydride derivative **36a** (scheme 5). Direct reduction of

36a using $LiAlH_4$ afforded **15a** as the main regioisomer.[29] For the regioselective formation of **14a** a different procedure via DCA-salt **37a** (scheme 5) had to be followed, the essence of which has been described for the preparation of **7a** (scheme 5, R=Me).[30] The moderate overall yield of **14a** is most likely due to concomitant 1,4-reduction of **38a**, a problem which was also encountered during the reduction of **36a** using selectride reagents.[31]

Scheme 5



a) 1. TFA 2. Et_3N, $ClCO_2Et$ b) $NaBH_4$ in THF/H_2O

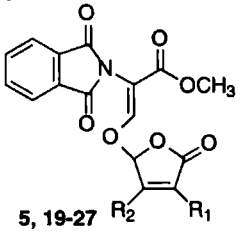
Biological evaluation

The germination stimulatory activity of phthalimido glycine derived D-ring analogs **5**, **19-30** and **35** was assayed using seeds of *Striga hermonthica* and *Orobanche crenata*. In each bioassay, GR24 (**4**) at an optimal concentration (0.01 mg/L for *Striga hermonthica* and 1 mg/L for *Orobanche crenata*) was included as a positive control. The last-mentioned compound enables a comparison between results obtained in different test series. This is important, since the respons of seeds of parasitic weeds, in particular of *Striga hermonthica*, varies considerably from test to test. The germination percentages thus obtained are collected in Tables 2a and 2b.

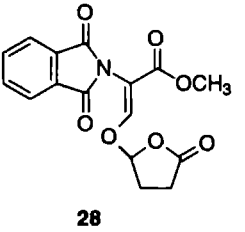
The data in Tables 2a and 2b reveal that most D-ring analogs exert little or no stimulatory effect. In particular seeds of *Orobanche crenata* are extremely sensitive toward structural modifications in the D-ring (Table 2b). Dimethyl substituted 2(5H)-furanone analog **22** shows a considerable degree of biological activity, and is at least as active as the parent compound **5** in the germination of seeds of *Striga hermonthica*. However, its activity in the stimulation of *Orobanche crenata* seed germination is only limited. Also α-ethyl 2(5H)-furanone derivative **24** gave significant stimulation of germination, particularly of seeds of *Striga hermonthica*. Its isomeric counterpart **35** was completely inactive, as expected because the D-ring is not a suitable leaving group (see molecular mechanism, scheme 1). For the same reason carba D-ring analog **29** does not exert germination inducing activity. A remarkable difference in activity was observed for β-methoxy substituted 2(5H)-furanone analog **23** and its β-methyl counterpart **22** (*cf.* entries 5 and 6, Table 2a), which, most likely, must be attributed to electronic factors. As a consequence,

the inherent reactivity of the α,β-unsaturated carbonyl function present in the D-ring should be taken into consideration as a potential recognition site of a receptor protein. Reduction of this highly reactive double bond (compound **28**) results in complete loss of activity. Strigol analogs containing 3-phenyl-, 4-phenyl- and 3,4-dichloro-2(5H)-furanone moieties as D-ring have previously been assayed as germination stimulants.[32] It was concluded that these compounds exhibit no stimulatory activity, although no data were provided. These results are confirmed here, using the related analogs **25-27**.

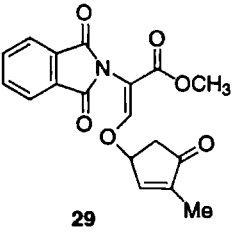
Table 2a. Germination percentages for seeds of *Striga hermonthica* after exposure to aqueous solutions of strigol analogs **5**, **19-30** and **35** at concentrations of 1 mg/L and 0.01 mg/L[a]



5, 19-27



28



29

		% germination ± S.E.		
entry	compound	1 mg/L	0.01 mg/L	**4** (0.01 mg/L)[b]
1	**5**, R_1=Me, R_2=H	37.3±5.0	n.a.	45.0±5.4
2	**19**, R_1-R_2=-$(CH)_4$-	n.a.	n.a.	45.0±5.4
3	**20**, R_1=H, R_2=H	13.1±1.9	n.a.	45.0±5.4
4	**21**, R_1=H, R_2=Me	27.4±3.6	n.a.	45.0±5.4
5	**22**, R_1=Me, R_2=Me	70.4±3.4	n.a.	73.3±2.1
6	**23**, R_1=Me, R_2=OMe	16.9±1.8	n.a.	73.3±2.1
7	**24**, R_1=Et, R_2=H	51.2±2.8	n.a.	61.6±3.6
8	**25**, R_1=Ph, R_2=H	n.a.	n.a.	45.3±5.1
9	**26**, R_1=H, R_2=Ph	n.a.	n.a.	45.3±5.1
10	**27**, R_1=Cl, R_2=Cl	n.a.	n.a.	45.3±5.1
11	**28**	n.a.	n.a.	50.4±4.4
12	**29**	n.a.	n.a.	73.3±2.1
13	**30**	n.a.	n.a.	61.3±3.7
14	**35**	n.a.	n.a.	61.6±3.6

[a] Activities are indicated as germination percentages obtained after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 550 seeds, distributed over 12 discs, of one representative experiment

[b] Values are the mean germination percentages ± S.E. obtained by treatment of the seeds with GR24 **4** (0.01 mg/L) in the same bioassay

n.a. No activity. Values are not significantly different from germination percentages obtained in the aqueous control

Table 2b. Germination percentages for seeds of *Orobanche crenata* after exposure to aqueous solutions of strigol analogs **5**, **19-30** and **35** at concentrations of 1 mg/L and 0.01 mg/L[a]

		% germination ± S.E.		
entry	compound	1 mg/L	0.01 mg/L	**4** (1 mg/L)[b]
1	**5**[c]	42.6±1.8	n.a.	66.1±1.8
2	**22**[d]	15.1±1.3	n.a.	66.1±1.8
3	**24**	14.9±1.0	n.a.	57.8±1.5
4	**19-21, 23, 25-30, 35**	n.a.	n.a.	51.7-74.9%

a Activities are indicated as germination percentages obtained after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 550 seeds, distributed over 12 discs, of one representative experiment

b Values are the mean germination percentages ± S.E. obtained by treatment of the seeds with GR24 **4** (1 mg/L) in the same bioassay

c germination percentage 58.3±1.3% at a concentration of 2 mg/L

d germination percentage 14.5±2.8% at a concentration of 2 mg/L

n.a. No activity. Values are not significantly different from germination percentages obtained in the aqueous control

9.3 Concluding remarks

Several D-ring analogs of strigol have been synthesized and assayed for germination stimulatory activity of seeds of *Striga hermonthica* and *Orobanche crenata*. It was found that the structure of the 2(5H)-furanone D-ring, which is present in all known strigolactones **1-3**, is very critical for the bioactivity. This finding suggests an essential role of the D-ring in the receptor interaction. From a practical point of view, replacement of the 3-methyl-2(5H)-furanone moiety by another D-ring is of considerable interest, as its preparation is a serious bottleneck in the large scale synthesis of GR24 and other strigol analogs. The slightly modified 3,4-dimethyl-2(5H)-furanone moiety is an attractive candidate for this purpose for the following reasons: First, the preparation of the corresponding 5-chloro synthon **11** can be readily performed in three steps from simple, cheap starting materials. Second, the coupling reaction with **11** proceeds in almost quantitative yield. Finally, the biological activity in the stimulation of *Striga hermonthica* seeds is hardly affected by this replacement of the common D-ring by the dimethyl congener. The use of **22**, or an analog containing a modified ABC-fragment, as a germination stimulant for the control of *Striga hermonthica* needs serious consideration and deserves further (field) studies.

9.4 Experimental section

Synthesis

General remarks

For general methods and instrumentation see chapter 2. IR spectra were recorded on Perkin-Elmer 298 infrared spectrophotometer. 90 MHz ^{1}H-NMR spectra were recorded on a Varian EM390 spectrometer.

Nijmegen 1 (**5**) was prepared as reported previously.[33] 2(5H)-Furanone **9a**,[34] 3-bromophthalide **8**[35] and 5-bromo-4-methyl-2(5H)-furanone **10**[8] were prepared following literature procedures.

5-Bromo-2(5H)-furanone (**9**)
2(5H)-Furanone **9a** was brominated with *N*-bromosuccinimide (NBS) according to the procedure of MacAlpine *et al.*[17] The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1) to give **9** as a yellow oil in 80% yield. ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 6.2 (d, 1H, J 6 Hz, =C$\underline{H}_\alpha$), 6.8 (d, 1H, J 1 Hz, C$\underline{H}$-Br), 7.62 (dd, 1H, J 6 Hz, J 1 Hz, =C$\underline{H}_\beta$) ppm.

5-Bromo-4-methoxy-3-methyl-2(5H)-furanone (**12**)
4-Methoxy-3-methyl-2(5H)-furanone **12a** (6.04 g, 47 mmol) was brominated with NBS according to the procedure of MacAlpine *et al.*[17] The crude product was purified by distillation to give **12** as a yellow oil in 55% yield. Bp 110-120^{0}C (3 mm); ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 2.0 (s, 3H, C$\underline{H}_3$), 4.1 (s, 3H, OC$\underline{H}_3$), 6.6 (s, 1H, C$\underline{H}$-Br) ppm.

5-Bromo-4-phenyl-2(5H)-furanone (**15**)
Precursor **15a** was prepared in 21% yield by reduction of phenylmaleic anhydride **36c** with lithium aluminium hydride according to the procedure of Kayser and Morand.[29] Bromination of **15a** with NBS following the procedure of MacAlpine *et al.*[17] afforded **15**[36] in quantitative yield and was used in the coupling reaction without further purification. ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 6.4 (s, 1H, =C$\underline{H}$), 7.2 (s, 1H, C$\underline{H}$-Br), 7.5 (m, 5H, arom. $\underline{H}$) ppm.

3-Phenyl-2(5H)-furanone (**14a**).
Phenylmaleic anhydride **36c** was prepared according to the method of Miller *et al.*[37] To a solution of phenylmaleic anhydride **36c** (3.24 g, 19.0 mmol) in methanol (15 mL) was gradually added dicyclohexylamine (DCA; 3.78 g, 21.0 mmol) with stirring at 0^{0}C. After 1 h the precipitate of the DCA-salt **39** was removed by filtration and washed with small portions of cold methanol to give 5.44 g (76%) of **39** as one single regioisomer according to ^{1}H-NMR analysis. ^{1}H-NMR ($CDCl_3$ + few drops of D_2O, 100 MHz): δ 1.21-2.12 (m, 20H, 20 cyclohexyl $\underline{H}$), 2.98 (m, 2H, 2 C$\underline{H}$-N), 3.82 (s, 3H, OC$\underline{H}_3$), 6.41 (s, 1H, =C$\underline{H}$), 7.36 (m, 5H, 5 arom. $\underline{H}$) ppm.
A solution of DCA-salt **39** (5.44 g, 14.0 mmol) in THF (10 mL) was treated with trifluoro acetic acid (TFA; 1.71 g, 15.0 mmol) at 0^{0}C. The TFA.DCA salt was removed by filtration. To the filtrate was gradually added triethylamine (1.4 g, 14 mmol) at 0^{0}C. The temperature was lowered to -30^{0}C and then ethyl chloroformate (1.6 g, 14 mmol) was added. The temperature was kept at -30^{0}C for 1 h after which the precipitate of Et_3N.HCl was filtered off. A solution of $NaBH_4$ (0.98 g, 26 mmol) in water (10 mL) was slowly added to the filtrate at 0^{0}C under vigorous stirring. The reaction mixture was stirred overnight at room temperature. The salts were removed by filtration and washed with diethyl ether. The filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to give **14a** as a greenish solid. Purification by recrystallization (2x) from hexane/benzene gave **14a** (0.54 g, 24%) as colorless plates. Mp 85^{0}C (lit. 89^{0}C).[38] ^{1}H-NMR data were in agreement with those reported previously.[29]

5-Bromo-3-phenyl-2(5H)-furanone (**14**)
Compound **14a** was brominated with NBS following the procedure of MacAlpine *et al.*[17] The reaction was stopped after 80% conversion to give a mixture of **14**:**14a** (4:1), which was used in

the coupling reaction without further purification. ^{1}H-NMR data of **14** were in complete agreement with those reported previously.[39]

3-Ethyl-3-phenylsulfanyl-dihydro-furan-2-one (**33**)
To a cooled solution (-78^{0}C) of freshly prepared lithium di*iso*propylamide (5.7 mmol) in THF (20 mL) was gradually added a solution of 3-phenylsulfanyl-dihydro-furan-2-one **32** (1.0 g, 5.2 mmol), prepared according to the procedure of Iwai *et al.*,[25] in THF (10 mL) After 15 min of stirring at -78^{0}C ethyl iodide (2.0 g, 14 mmol) was added and the reaction mixture was stirred overnight at room temperature. Saturated NH_4Cl was added and THF was removed *in vacuo* The residue was extracted with ethyl acetate (3x) The combined extracts were washed with water (2x), dried ($MgSO_4$) and concentrated *in vacuo* to give **33** (0.68 g, 60%) as an oil, which was used in the next reaction without further purification. Purity 95% according to GC, ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.1 (t, J 7.5 Hz, 3H, C$\underline{H}_3$), 1.9 (m, 2H, C$\underline{H}_2$), 2.2 (q, J 7.5 Hz, 2H, C$\underline{H}_2$CH$_3$), 4.2 (m, 2H, C$\underline{H}_2$-O), 7.2 (m, 5H, 5 arom $\underline{H}$) ppm.

3-Ethyl-2(5H)-furanone (**13a**)
A solution of **33** (8.3 g, 37.4 mmol) in dichloromethane (50 mL) at 0^{0}C was treated with 80% *m*CPBA (7.7 g, 45 mmol) in dichloromethane (30 mL). The reaction mixture was stirred at 0^{0}C for 1 h. Then a saturated solution of Na_2SO_3 (50 mL) was added and the organic phase was successively washed with satd. $NaHCO_3$, water and brine. Drying ($MgSO_4$) and concentration *in vacuo* afforded the crude sulfoxide in nearly quantitative yield. The residue was heated at reflux temperature in CCl_4 (50 mL) for 1 h. The solvent was removed under reduced pressure and the residue was purified by distillation to give **13a** (1 5 g, 40%) as a colorless oil. Bp 42^{0}C (0 1 mm); ^{1}H-NMR ($CDCl_3$, 90 MHz). δ 1 1 (t, J 6 Hz, 3H, C$\underline{H}_3$), 2 2 (m, 2H, C$\underline{H}_2$CH$_3$), 4.7 (m, 2H, C$\underline{H}_2$), 7 0 (m, 1H, =C$\underline{H}$) ppm.

Bromination of 3-ethyl-2(5H)-furanone (**13a**)
Compound **13a** (1 0 g, 8.9 mmol) was brominated with NBS according to the procedure of MacAlpine *et al* [17] A mixture of mainly **13** and **34** in an equimolar ratio was isolated in quantitative yield, which could not be separated. This mixture was used in the coupling reaction (*vide infra*).

4-Bromo-2-methyl-cyclopent-2-enone (**18**)
2-Methyl-cyclopent-2-enone **18a** was prepared as described by Gassman and Pascone.[21] The bromination reaction was performed similarly as reported by DePuy *et al.*[16] starting from **18a** (4.20 g, 43.8 mmol). Yield 5.51 g (72%) of **18** after distillation. Bp 52-55^{0}C (0 25 mm); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.85 (m, 3H, C$\underline{H}_3$), 2.73 (dd, J 2 Hz, ^{2}J 20 Hz, 1H, C$\underline{H}_2$), 3.08 (dd, J 6 Hz, ^{2}J 20 Hz, 1H, C$\underline{H}_2$), 5 11 (m, 1H, C$\underline{H}$-Br), 7.32 (m, 1H, =C$\underline{H}$) ppm.

Coupling of chloro- and bromofuranones ***7-15, 17*** *with Sheehan aldehyde* ***6*** *(general procedure)*
To a solution of Sheehan aldehyde **6** (10 mmol) in DMF (50 mL) was added potassium *tert*-butoxide (11 mmol) in a nitrogen atmosphere. The mixture was cooled to -60^{0}C and a solution of the chloro- or bromofuranone **8-15**, **17** (11 mmol) in DMF (10 mL) was added with stirring Stirring was continued for 18 h at room temperature. Then, DMF was removed *in vacuo* and the residue was dissolved in a mixture of water and ethyl acetate. The aqueous layer was extracted with ethyl acetate (3x) and the combined organic extracts were washed with water (2x), dried

($MgSO_4$) and concentrated *in vacuo* The crude coupling products **19-26, 28** were further purified by flash chromatography and/or crystallization

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(3-oxo-1,3-dihydro-isobenzofuran-1-yloxy)-acrylate (**19**)
Coupling of 3-bromophthalide **8** with Sheehan aldehyde **6** (2 5 g, 10 mmol) was carried out as described in the general procedure The crude product was purified by crystallization from 2-propanol to give **19** (2 1 g, 55%) as white crystals Mp 156-160^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 3 78 (s, 3H, $OC\underline{H}_3$), 6 72 (br s, 1H, $OC\underline{H}O$), 7 70-7 90 (m, 8H, 8 arom $\underline{H}$), 7 97 (s, 1H, $=C\underline{H}O$) ppm, Analysis calcd for $C_{20}H_{13}NO_7$ C, 63 33, H, 3 45, N, 3 69 Found C, 62 92, H, 3 43, N, 3 64

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**20**)
Coupling of 5-bromo-2(5H)-furanone **9** with Sheehan aldehyde **6** (2 5 g, 10 mmol) was carried out as described in the general procedure The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1 1) to give **20** (1 2 g, 37%) as a white solid An analytical sample was obtained by crystallization from 2-propanol Mp 146-148^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 3 78 (s, 3H, $OC\underline{H}_3$), 6 33 (br s, 1H, $OC\underline{H}O$), 6 37 (dm, J 5 Hz, 1H, $=C\underline{H}_\alpha$), 7 34 (dm, J 5 Hz, $=C\underline{H}_\beta$), 7 72-7 96 (m, 5H, 4 arom $\underline{H}$ + $=C\underline{H}O$) ppm, MS (EI, m/z, rel int (%)) 329 ($[M]^+$, 1 5), 246 ($[C_{12}H_8NO_5]^+$, 100), 83 ($[C_4H_3O_2]^+$, 37 5), Analysis calcd for $C_{16}H_{11}NO_7$ C, 59 77, H, 4 24, N, 5 36 Found C, 60 53, H, 4 33, N, 5 56

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol 2-yl)-3-(3-methyl-5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**21**)
Coupling of 5-bromo-4-methyl-2(5H)-furanone **10** with Sheehan aldehyde **6** (1 23 g, 5 00 mmol) was carried out as described in the general procedure The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1 1), followed by crystallization from 2-propanol to give **21** (200 mg, 12%) as white crystals Mp 170-173^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 2 11 (d, 3H, ^{4}J 1 4 Hz, $C\underline{H}_3$), 3 79 (s, 3H, $OC\underline{H}_3$), 5 99 (m, 1H, $=C\underline{H}$), 6 08 (br s, 1H, $OC\underline{H}O$), 7 72-7 96 (m, 5H, 4 arom $\underline{H}$ + $=C\underline{H}O$) ppm, Analysis calcd for $C_{17}H_{13}NO_7$ C, 59 48, H, 3 82, N, 4 08 Found C, 59 32, H, 3 83, N, 4 04

Methyl 3 (3,4-dimethyl-5-oxo-2,5-dihydro-furan-2-yloxy)-2-(1,3-dioxo 1,3-dihydro-isoindol-2-yl)-acrylate (**22**)
Coupling of 5-chloro-3,4-dimethyl-2(5H)-furanone **11** with Sheehan aldehyde **6** (1 24 g, 5 00 mmol) was carried out as described in the general procedure The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1 2) to give **22** (1 75 g, 95%) as a slightly yellow solid An analytical sample was obtained by crystallization from di*iso*-propyl ether/ethyl acetate Mp 155-157^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 1 85 (m, 3H, $C\underline{H}_3$), 1 99 (m, 3H, $C\underline{H}_3$), 3 78 (s, 3H, $OC\underline{H}_3$), 6 02 (br s, 1H, $OC\underline{H}O$), 7 92 (m, 5H, 4 arom $\underline{H}$ + $=C\underline{H}O$) ppm, MS (EI, m/z, rel int (%)) 357 ($[M]^+$, 1 8), 246 ($[C_{12}H_8NO_5]^+$, 57 9), 111 ($[C_6H_7O_2]^+$, 100), Analysis calcd for $C_{18}H_{15}NO_7$ C, 60 51, H, 4 23, N, 3 92 Found C, 60 55, H, 4 16, N, 3 85

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(3-methoxy-4-methyl-5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**23**)
Coupling of 5-bromo-4-methoxy-3-methyl-2(5H)-furanone **12** with Sheehan aldehyde **6** (1.20 g, 4.90 mmol) was carried out as described in the general procedure. The crude product (1.55 g, 85%, purity according to ^{1}H-NMR > 90%) was crystallized from 2-propanol to give **23** (1.16 g, 63%) as colorless crystals. Mp 173^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.89 (d, ^4J < 1 Hz, 3H, $C\underline{H}_3$), 3.78 (s, 3H, $OC\underline{H}_3$), 4.05 (s, 3H, $=COC\underline{H}_3$), 6.00 (d, ^4J < 1 Hz, 1H, $OC\underline{H}O$), 7.78 (s, 1H, $=C\underline{H}O$), 7.73-7.97 (m, 4H, 4 arom. H) ppm; MS (EI, m/z, rel. int. (%)): 373 ([M]$^+$, 0.9), 246 ([$C_{12}H_8NO_5$]$^+$, 5.2), 127 ([$C_6H_7O_3$]$^+$, 100); Analysis calcd for $C_{18}H_{15}NO_8$: C, 57.91; H, 4.05; N, 3.75. Found: C, 58.07; H, 4.04; N, 3.76.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-ethyl-5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**24**) *and methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-[1-(2-oxo-2,5-dihydro-furan-3-yl)-ethoxy]-acrylate* (**35**)
Sheehan aldehyde **6** (1.1 g, 4.5 mmol) was treated with the crude mixture, obtained from bromination of 3-ethyl-2(5H)-furanone **13a** (8.9 mmol), according to the general procedure. Purification was accomplished by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) to give coupling products **24** (250 mg, 16%) and **35** (370 mg, 23%) as white solids. Analytical samples of **24** and **35** were obtained by crystallization from ethanol and *n*-butyl acetate, respectively.
Compound **24**: Mp 164^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.17 (t, J 7.4 Hz, 3H, $CH_2C\underline{H}_3$), 2.35 (br q, J 7.4 Hz, 2H, $C\underline{H}_2CH_3$), 3.79 (s, 3H, $OC\underline{H}_3$), 6.21 (m, 1H, $OC\underline{H}O$), 6.86 (m, 1H, $=C\underline{H}$), 7.78 (s, 1H, $=C\underline{H}O$), 7.72-7.95 (m, 4H, 4 arom. H) ppm; MS (EI, m/z, rel. int. (%)): 357 ([M]$^+$, 2.1), 246 ([$C_{12}H_8NO_5$]$^+$, 100), 111 ([$C_6H_7O_2$]$^+$, 29); Analysis calcd for $C_{18}H_{15}NO_7$: C, 60.51; H, 4.23; N, 3.92. Found: C, 60.26; H, 4.22; N, 3.91.
Compound **35**: Mp 162-164^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.54 (d, J 6.4 Hz, 3H, $C\underline{H}_3$), 3.76 (s, 3H, $OC\underline{H}_3$), 4.90 (m, 2H, $OC\underline{H}_2$), 5.00 (m, 1H, $C\underline{H}CH_3$), 7.67-7.97 (m, 6H, 4 arom. H, $=C\underline{H}$, $=C\underline{H}O$) ppm; Analysis calcd for $C_{18}H_{15}NO_7$: C, 60.51; H, 4.23; N, 3.92. Found: C, 60.26; H, 4.20; N, 4.17.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-phenyl-5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**25**)
Coupling of 5-bromo-3-phenyl-2(5H)-furanone **14** with Sheehan aldehyde **6** (358 mg, 1.45 mmol) was carried out as described in the general procedure. The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) to give **25** (122 mg, 21%) as a white solid. An analytical sample was obtained by crystallization from 2-propanol. Mp 171-172^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 3.79 (s, 3H, $OC\underline{H}_3$), 6.33 (d, J 1.6 Hz, 1H, $OC\underline{H}O$), 7.35-7.46 (m, 4H, 3 arom. H + $=C\underline{H}$), 7.70-7.95 (m, 6H, 6 arom. H), 7.98 (s, 1H, $=C\underline{H}O$) ppm; MS (EI, m/z, rel. int. (%)): 405 ([M]$^+$, 1.7), 246 ([$C_{12}H_8NO_5$]$^+$, 81.7), 159 ([$C_{10}H_7O_2$]$^+$, 100); Analysis calcd for $C_{22}H_{15}NO_7$: C, 65.19; H, 3.73; N, 3.46. Found: C, 64.84; H, 3.79; N, 3.46.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(3-phenyl-5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**26**)
Coupling of 5-bromo-4-phenyl-2(5H)-furanone **15**[36] with Sheehan aldehyde **6** (598 mg, 2.42 mmol) was carried out as described in the general procedure. The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1) to give **26** (262 mg, 27%) as a white solid. Analytically pure **26** was obtained by crystallization from 2-propanol. Mp 142-144^{0}C; ^{1}H-NMR

($CDCl_3$, 100 MHz)· δ 3 77 (s, 3H, $OC\underline{H}_3$), 6.51 (s, 1H, $OC\underline{H}O$)*, 6 61 (s, 1H, =C<u>H</u>)*, 7 50 (m, 5H, 5 arom <u>H</u>), 7.78 (m, 4H, 4 arom. <u>H</u>), 8.04 (s, 1H, =C<u>H</u>O) ppm, (*: signals may be interchanged); MS (EI, m/z, rel. int. (%)): 405 ([M]$^+$, 1.0), 246 ([$C_{12}H_8NO_5$]$^+$, 38.6), 159 ([$C_{10}H_7O_2$]$^+$, 100); Analysis calcd for $C_{22}H_{15}NO_7$: C, 65.19; H, 3.73; N, 3.46. Found: C, 64.33; H, 3.70; N, 3.51.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(5-oxo-tetrahydro-furan-2-yloxy)-acrylate (**28**)
Coupling of 5-chloro-γ-butyrolactone **17** with Sheehan aldehyde **6** (1.62 g, 6.56 mmol) was carried out as described in the general procedure. The crude product was purified by recrystallization from 2-propanol to give **28** (1.07 g, 49%) as colorless crystals. Analytically pure **28** was obtained by repeated crystallization from 2-propanol. Mp 162-164^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz). δ 2.28-2 73 (m, 4H, 2 $C\underline{H}_2$), 3 78 (s, 3H, $OC\underline{H}_3$), 5 97 (m, 1H, $OC\underline{H}O$), 7.72-7.97 (m, 5H, 4 arom <u>H</u> + =C<u>H</u>O) ppm; MS (CI, m/z, rel. int. (%)). 332 ([M+1]$^+$, 2.2), 247 ([$C_{12}H_9NO_5$]$^+$, 61), 85 ([$C_4H_5O_2$]$^+$, 100); Analysis calcd for $C_{16}H_{13}NO_7$· C, 58.01; H, 3.96; N, 4.23. Found. C, 57 88, H, 3.85, N, 4.23.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(3-methyl-4-oxo-cyclopent-2-enyloxy)-acrylate (**29**)
This compound was prepared in a similar way as described for the synthesis of furanones **19-26**, starting from Sheehan aldehyde **6** (1 62 g, 6.56 mmol) and 4-bromo-2-methyl-cyclopent-2-enone **18** (1 15 g, 6 56 mmol). ^{1}H-NMR analysis of the crude product (2.1 g) revealed the presence of an equimolar ratio of **29** and cyclopentadienone dimers **31**. Trituration (di*iso*propyl ether) provided a solid (1.4 g), consisting of unreacted **6** and coupling product **29**. Crystallization from di*iso*propyl ether/ethyl acetate afforded analytically pure **29** (325 mg, 15%) as colorless crystals. Mp 183-184^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz). δ 1.83 (m, 3H, $C\underline{H}_3$), 2 45 (dd, J 2.1 Hz, ^{2}J 18 7 Hz, 1H, $C\underline{H}_2$), 2.89 (dd, J 6.1 Hz, ^{2}J 18.7 Hz, 1H, $C\underline{H}_2$), 3.76 (s, 3H, $OC\underline{H}_3$), 5.28 (m, 1H, $OC\underline{H}CH_2$), 7.18 (m, 1H, =C<u>H</u>), 7 70-7.95 (m, 5H, 4 arom. <u>H</u> + =C<u>H</u>O) ppm, MS (EI, m/z, rel. int (%))· 341 ([M]$^+$, 3 2), 247 ([$C_{12}H_9NO_5$]$^+$, 41.9), 95 ([C_6H_7O]$^+$, 100); Analysis calcd for $C_{18}H_{15}NO_6$. C, 63.34; H, 4.43, N, 4.10. Found: C, 63.21; H, 4.28; N, 4.13.

*DCC-coupling of Sheehan aldehyde **6** and mucochloric acid **16***
Procedure a. Synthesis of *methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-[2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl-2-methoxycarbonyl-vinyloxy]-acrylate* (**30**)
Mucochloric acid **16** (3 4 g, 20 mmol) was suspended in dichloromethane (20 mL), followed by addition of Sheehan aldehyde **6** (4 9 g, 20 mmol). At 0^{0}C, dicyclohexyl carbodiimide (DCC; 4 1 g, 20 mmol) was added and the suspension was stirred for 15 h at room temperature. The precipitate (DCU) was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was recrystallized from 2-propanol to give the dimer **30** (3.0 g, 63%) as colorless crystals. An analytical sample was obtained by crystallization from acetic acid Mp > 230^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 3.79 (s, 6H, 2 $OC\underline{H}_3$), 7.61 (s, 8H, 8 arom. <u>H</u>), 7.93 (s, 2H, 2 =C<u>H</u>O), MS (EI, m/z, rel int (%)). 476 ([M]$^+$, 13.3), 416 ([$C_{22}H_{12}N_2O_7$]$^+$, 29.3), 230 ([$C_{12}H_8NO_4$]$^+$, 100), Analysis calcd for $C_{24}H_{16}N_2O_9$: C, 60.51; H, 3.38; N, 5.88. Found: C, 60.27; H, 3.35; N, 5.82.
Procedure b: Synthesis of *methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-[1-(2-oxo-2,5-dihydro-furan-3-yl)-ethoxy]-acrylate* (**27**)
To a cooled (-5^{0}C) solution of Sheehan aldehyde **6** (2 5 g, 10 mmol) and mucochloric acid (1 7 g, 10 mmol) in THF (10 mL) in the presence of a catalytic amount of DMAP was added DCC (2 2

g, 10 mmol). A precipitate of DCU gradually formed. After 18 h of stirring at room temperature the precipitate was removed by filtration. The residue was concentrated *in vacuo*. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) afforded **27** (1.4 g, 35%) as a white solid. An analytical sample was obtained by crystallization from acetic acid. Mp 157^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 3.80 (s, 3H, OC<u>H</u>$_3$), 6.18 (s, 1H, OC<u>H</u>O), 7.73-7.97 (m, 5H, 4 arom. <u>H</u> + =C<u>H</u>O); MS (EI, m/z, rel. int. (%)): 399 ([$C_{16}H_9{}^{35}Cl^{37}ClNO_7$]$^+$, 0.7), 397 ([$C_{16}H_9{}^{35}Cl_2NO_7$]$^+$, 1.1), 368 ([$C_{15}H_6{}^{35}Cl^{37}ClNO_6$]$^+$, 1.0), 366 ([$C_{15}H_6{}^{35}Cl_2NO_6$]$^+$, 1.6), 246 ([$C_{12}H_8NO_5$]$^+$, 100), 153 ([$C_4H^{35}Cl^{37}ClO_2$]$^+$, 9.2), 151 ([$C_4H^{35}Cl_2O_2$]$^+$, 14.4); Analysis calcd for $C_{16}H_9Cl_2NO_6$: C, 48.27; H, 2.28; N, 3.52. Found: C, 48.13; H, 2.26; N, 3.44.

Biological activity

Seeds.

Seeds of *Striga hermonthica* (from *Sorghum bicolor* (L.) Moench) and *Orobanche crenata* (from *Vicia faba* L.) were harvested in Sudan in 1987 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of test solutions.

A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a. and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1 and 0.01 mg/L test compound and 0.1 and 0.001% (v/v) acetone, respectively.

Bioassays

For surface sterilization seeds of *Striga hermonthica* and *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30-70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20^{0}C for *Orobanche* seeds and at 30^{0}C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.1 and 0.001% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (concentrations of 1 and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks per treatment.

For full details of the bioassay, see Mangnus *et al.*[40]

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Chapter 10*

Synthesis and Biological Evaluation of the Strigol Analog Carba GR24

Abstract: Both geometrical isomers of the strigol analog carba-GR24 (**1**) (*E*-isomer) and (**7**) (*Z*-isomer) were prepared These analogs, in which the vinyl ether oxygen atom in GR24 has been replaced by a methylene function, are completely inactive in the stimulation of germination of seeds of the parasitic weeds *Striga hermonthica* and *Orobanche crenata* Bioassays in which **1** was tested as an inhibitor of germination, gave no indication as such

10.1 Introduction

A few naturally occurring compounds, *viz* (+)-strigol,[1-3] sorgolactone[4] and alectrol[5] (collectively called 'strigolactones')[6] have been recognized as highly active germination stimulants of seeds of the parasitic weeds *Striga* and *Orobanche*

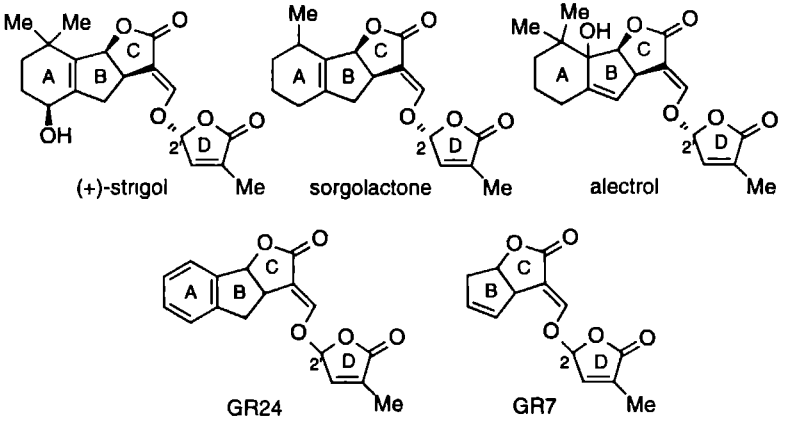

Figure 1

Extensive structure-activity studies revealed that the bioactiphore resides in the CD-part of these molecules [7-10] Synthetic analogs, especially the so-called GR compounds GR24 and GR7 (fig 1) received much attention [11,12] These compounds were designed by systematically

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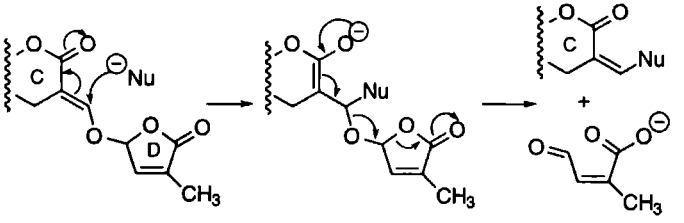
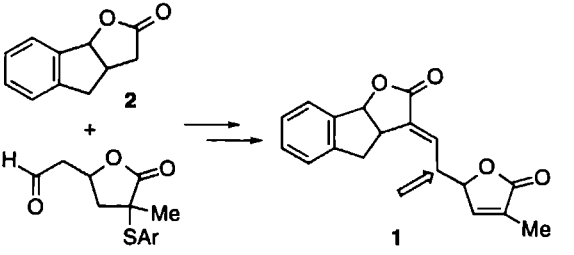
removing parts of the strigol skeleton. From the GR series, GR24 (fig.1) is the most potent one, whose half-maximal activity is at 10^{-9}M (*Striga hermonthica*), which is comparable to that of strigol. Recently, a tentative molecular mechanism has been proposed, which may account for the biological activity of strigol and its (synthetic) analogs.[13] This mechanism, which involves only the bioactiphoric CD-part is depicted in scheme 1.

Scheme 1



The enol ether unit plays a crucial role in this mechanism, since it enables the D-ring to eliminate as is shown. Replacement of the enol ether oxygen atom by the bioisosteric methylene group will provide valuable, additional information about the bioactiphore. The strategy for the synthesis of carba-GR24 (**1**) was reported recently.[14] The key step involves coupling of the ABC moiety **2** and the latent D-ring fragment **3a** (scheme 2). The previously described coupling method, which involves essentially the procedure of Tanaka[15] proved to be rather problematic. Therefore, another method for the coupling was considered. The primary aim of the work described in this chapter is to establish the germinating ability of carba-GR24.

Scheme 2



3a, Ar=Ph
3b, Ar=Ph-Cl(*p*)

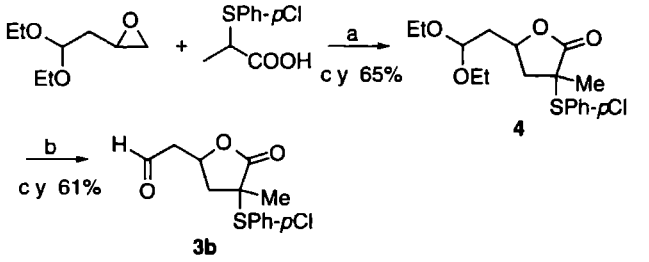
10.2 Results and discussion

Synthesis

The key step in the synthesis of carba GR24 (**1**) proceeds by coupling of tricyclic lactone **2** with an appropiate latent butenolide fragment (scheme 2). It is essential to use this protected D-

ring synthon, because the corresponding butenolide is too unstable to survive the coupling conditions Previously the phenylsulfanyl group as protecting function in the D-ring synthon was used During the revision of the coupling step the *p*-chlorophenylsulfanyl moiety was considered as an alternative, because this group would probably facilitate crystallization of intermediate products The D-ring synthon **3b** was prepared essentially in the same manner as reported earlier, from 2-(2,2-diethoxyethyl)oxirane and α-(*p*-chlorophenylsulfanyl)propionic acid, and subsequent hydrolysis of the acetal function (scheme 3) This sequence led to the formation of **3b** as a mixture of diastereomers in a ratio of 2 1 No attempts were undertaken to separate these products Aldehyde **3b**, thus obtained, is a rather unstable compound and has to be used immediately in the subsequent step, its quality after chromatography (florisil) varied to some extent

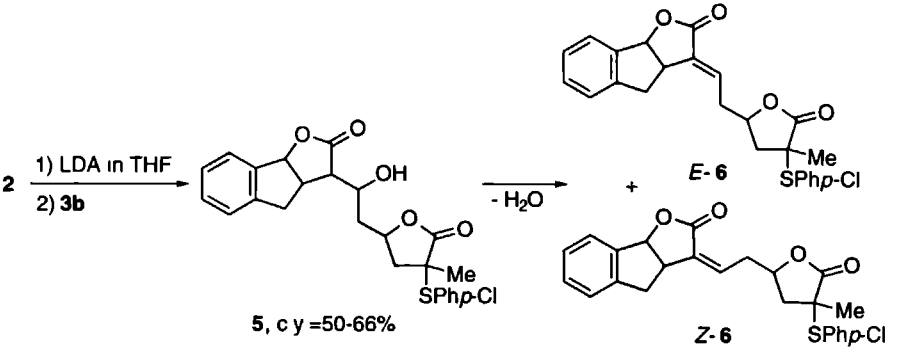
Scheme 3



a) 1 LDA (2 equiv) 2 aq tartaric acid 3 SiO_2, Δ b) 0 5 N HCl, Δ

Aldol condensation of aldehyde **3b** with ABC-fragment **2** gave product **5** as a complex mixture of diastereomers (scheme 4) From this mixture, one diastereomer was obtained in a pure form by flash-chromatography and recrystallization The relative configuration of this single diastereomer has not been established The subsequent dehydration step (scheme 4) was not as straightforward as expected

Scheme 4



Several conventional methods failed to give the desired result, e.g. *p*-toluenesulfonic acid catalysis, Ac_2O-Δ, *p*-TosCl-DBU, $POCl_3$-HMPA-pyridine,[16] DCC-CuCl.[17,18] Only mesylation (MsCl, Et_3N), followed by elimination (DBU) gave the desired transformation, whereby the *Z*-isomer *Z*-**6** was obtained as the main product. A photochemical isomerization of the exocyclic double bond to the *E*-geometry could be accomplished, however this is not recommendable since an extra step and an extra chromatographic separation are required. This step could indeed be avoided when aldol **5** was treated with 2-fluoro-*N*-methylpyridinium *p*-toluenesulfonate in the presence of Et_3N.[19,20] Under these conditions the *E*-isomer *E*-**6** was formed predominantly. These findings were a reason to investigate the dehydration reaction in more detail. Relevant results are collected in Table I. Because the relative configuration of diastereomers **5** is unknown, they are indicated by their chromatographic behavior on silica gel.

Table I. Product distribution after exposing two diastereomeric compounds **5** to different elimination conditions

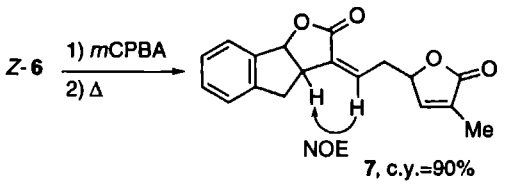
R_f aldol **5**[a]	yield of **6**[b]	E:Z ratio[b]	yield of **6**[c]	E:Z ratio[c]
0.17	72%	only Z	95%	2:1
0.06	30%	1:1	90%	only E

a) R_f-value on TLC (SiO_2, eluens hexane/ethyl acetate=2/1)
b) prepared by mesylation, followed by DBU treatment
c) prepared by treatment with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate and Et_3N

The choice of the starting diastereomer is an important factor for the yield of the reaction as well as its stereochemical outcome. Mesylation and subsequent treatment with DBU generally leads to the preponderant formation of the *Z*-isomer, whereas reaction with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate and Et_3N predominantly leads to the *E*-isomer. The latter method gave a cleaner reaction and higher yields. Starting from a mixture of diastereomers either selected method led, on a preparative scale, to the expected geometrical isomer *E*-**6** and *Z*-**6** in isolated yields ranging from 45-70%. These results indicate that the elimination reactions take place via different mechanisms. Amberg and Seebach[21] suggested a *syn*-type mechanism for a similar base-induced elimination of a mesylate, which can be envisaged by deprotonation of the mesyl methyl group and a subsequent *syn* proton abstraction via a cyclic transition state. This explanation is in agreement with the observation that elimination of the corresponding tosylate under the same conditions does not take place smoothly, but rather leads to an undefined mixture of products. The formation of *E*-**6** by elimination of the *N*-methylpyridinium salt proceeds in a anti-periplanar fashion.

Finally, removal of the protecting thioether moiety was carried out by oxidation to the corresponding sulfoxides and their subsequent pyrolysis to give in high yields *Z*-carba-GR24 (**7**) and *E*-carba-GR24 (**1**), both as mixtures of two diastereomers (scheme 5), which could not be separated by chromatography.

Scheme 5



Similarly: *E*-**6** ⟶ **1**, c.y.=94%

The assignment of the E/Z geometry was based on their ^{1}H-NMR spectra. The signal of the exocyclic vinylic proton for the *E*-isomer was about 0.3 ppm further downfield as compared to the corresponding signal for the *Z*-adduct (anisotropic effect of the carbonyl). In addition, a NOE was observed between the exocyclic vinylic proton and H_{3a} in the 2D-NOESY spectrum of the *Z*-isomer (MM2 calculated distance 2.74 Å), which was absent for the *E*-isomer (MM2 calculated distance 3.92 Å).

Biological activity

The stimulatory activity of the carba GR24 analogs **1** and **7** was assayed using seeds of *Striga hermonthica* and *Orobanche crenata*. The germination percentages are collected in Table II, together with those obtained for GR24 under the same conditions in the same bioassay. (This enables a comparison between results obtained in different test series, which is important, because the respons of seeds of parasitic weeds, in particular of *Striga hermonthica*, varies considerably from test to test). For the evaluation of the bioactivity mixtures of diastereomers were used, which is acceptable because it was shown for GR24 that the activity of the most active diastereomer is hardly influenced by the presence of the less active diastereomer.[22]

Table II. Germination percentages for seeds of *Striga hermonthica* and *Orobanche crenata* after exposure to solutions of **1** and **7**[a]

	Striga hermonthica		*Orobanche crenata*	
	% germination ±S.E. at		% germination ±S.E. at	
compound	1 mg/L	0.01 mg/L	1 mg/L	0.1 mg/L
1	2.73±1.52	2.94±1.03	0.0±0	0.0±0
	(37.7±3.5)[b]	(55.1±2.6)[b]	(59.9±3.2)[b]	22.1±(1.4)[b]
7	2.11±0.60	3.78±0.89	0.0±0	0.0±0
	(50.5±4.2)[b]	(67.5±0.4)[b]	(59.9±3.2)[b]	22.1±(1.4)[b]

a) Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 550 seeds, distributed over 12 discs, of one representative experiment.

b) The values in parantheses are the mean germination percentages for seeds tested under the same conditions and at the same time, with GR24 as stimulant

The results indicate that *E* and *Z* carba-GR24 (**1**) and (**7**) are completely inactive in the stimulation of seed germination of *Striga hermonthica* and *Orobanche crenata*. This means that isosteric replacement of oxygen in GR24 by carbon causes complete loss of activity. This finding supports the tentative molecular mechanism for the stimulation of germination, as proposed in scheme 1. These results can be explained by assuming either that both carba-analogs do not fit properly in the receptor cavity for either steric or electronic reasons, or that the affinity is sufficiently high, but it does not lead to a transduction of a signal. According to the proposed molecular mechanism (scheme 1), essentially two events take place within the receptor site. The addition to the α,β-unsaturated system in a Michael fashion may still occur in case of carba-GR24. The subsequent elimination reaction, however, is prevented, because the D-ring is not a leaving group anymore. The essence of the elimination of the D-ring remains to be established, but it may well be possible that the D-ring, which is a highly reactive species, fulfills the function of a second messenger.

It may be hypothesized that carba-GR24 acts as an inhibitor of the germination process by assuming that carba GR24 acts as a Michael acceptor in the same way as is proposed for GR24 (scheme 1). However, the active site of the receptor is now being blocked and an inhibitory effect is expected. It may also be argued that the inherent chemical reactivity of the α,β-unsaturated system in carba GR24 does not play a role. In that case competition of carba GR24 and GR24 for the same receptor site with a comparable degree of affinity, may still lead to a concentration dependent inhibitory effect. In order to verify these hypotheses, two types of experiments were conducted employing seeds of *Striga hermonthica*. In both experiments *E*-carba-GR24 **1** was used as a potential inhibitor. In the first experiment preconditioned seeds of *Striga hermonthica* were preincubated for 24 h in solutions containing different concentrations of **1**. The solution of **1** was then carefully removed, the seeds were washed several times and then treated with different concentrations of GR24. The results are presented in fig 2. These data reveal that **1** does not act as an irreversible inhibitor of germination.

In order to find evidence for a competitive mode of inhibition, a different experiment was conducted. In this experiment preconditioned seeds of *Striga hermonthica* were incubated in the presence of mixtures of GR24 and **1** at different concentrations, the results of which are depicted in fig 3. Again, no inhibitory effect of **1** is observed.

From figures 2 and 3 it may be concluded that *E*-carba-GR24 **1** does not exert any inhibitory effect. Apparently, the lack of stimulatory activity of **1** cannot be attributed to (irreversible) blocking of the receptor site through its Michael acceptor, nor does **1** possess an affinity for the receptor comparable to that of GR24.

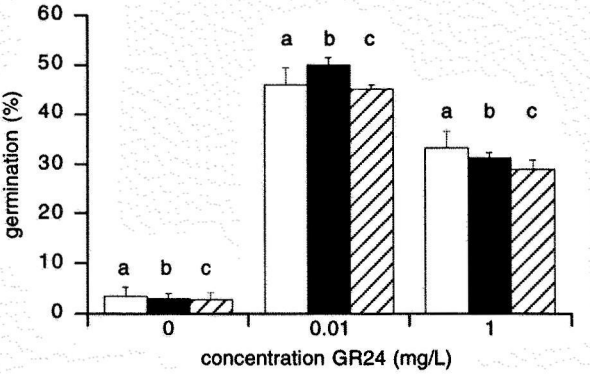


Figure 2. Effect of *E*-carba-GR24 **1** on the germination stimulatory activity of GR24 . Preconditioned seeds of *Striga hermonthica* were incubated with solutions of **1** for 24h. After removal of **1**, the seeds were exposed to GR24 at the indicated concentrations. a) Seeds preincubated with aqueous control, followed by treatment with GR24, b) Seeds preincubated with **1** (0.01 ppm), followed by treatment with GR24, c) Seeds preincubated with **1** (1 ppm), followed by treatment with GR24. Data presented ± S.E. are from one representative experiment.

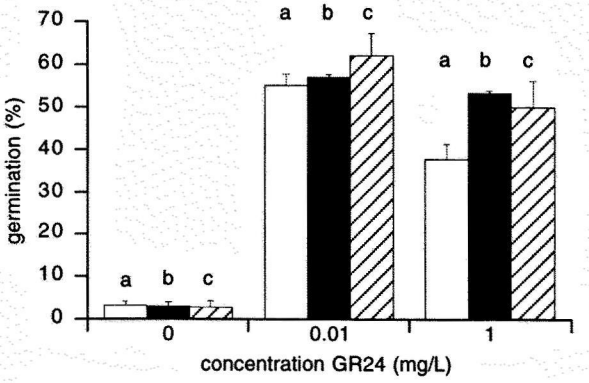


Figure 3. Effect of *E*-carba-GR24 **1** on the germination stimulatory activity of GR24 . Preconditioned seeds of *Striga hermonthica* were treated with a) only GR24 b) mixtures of *E*-carba-GR24 (0.01 ppm) and GR24 c) mixtures of *E*-carba-GR24 (1 ppm) and GR24 at the indicated concentrations. Data presented ± S.E. are from one representative experiment.

10.3 Concluding remarks

An improved synthesis of *Z*- and *E*-carba-GR24 is presented. The biological data indicate that isosteric replacement of oxygen by carbon causes complete loss of stimulatory activity. Moreover, no inhibitory effect was observed for *E*-carba-GR24 **1**. It should be emphasized that so far no strigol analogs were designed and assayed for inhibitory activity, for reasons that assays for stimulant activity are much easier to perform and to interpret than assays for inhibitor activity. These data, nevertheless, are essential since they may provide valuable information about the molecular basis of the germination process. It should be noted that compounds specific for inhibition of *Striga* and *Orobanche* seed germination would serve practical purposes in the eradication of these parasitic weeds.[6,23]

10.4 Experimental section

Synthesis

General remarks

100 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard) and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me_4Si as internal standard). All coupling constants are given as ^3J in Hz, unless indicated otherwise. For mass spectra a double focussing VG7070E mass spectrometer was used. GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30m x 0.25 mm). Helium was used as carrier gas, and electron impact (EI) was used as ionization mode. GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.

Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH_2. Tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0.25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray. Flash-chromatography was carried out at a pressure of *ca.* 1.5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was carried out, using Merck Kieselgel 60.

3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one (**2**) was prepared following a previously reported synthesis.[22]

[4-(4-Chloro-phenylsulfanyl)-4-methyl-5-oxo-tetrahydrofuran-2-yl]-acetaldehyde (**3b**).

Precursor **4** was prepared similarly as described for the synthesis of 5-(2,2,-diethoxyethyl)-3-methyl-3-(phenylthio)-dihydrofuran-2-one.[14] Yield after chromatography (SiO_2, hexane/ethyl acetate 3:1) 65% as a yellow oil. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.22 (2t, 6H, J 7.5 Hz, $(OCH_2\underline{CH_3})_2$), 1.47, 1.52 (2s, 3H, $C\underline{H_3}$), 1.79-2.21 (m, 3H, $\underline{CH_2}CH(OEt)_2$, $C\underline{H_2}$), 2.35-2.60 (m,

1H, C$\underline{H}_2$), 3.58 (m, 4H, (OC$\underline{H}_2$CH$_3$)$_2$), 4.63 (m, 2H, C$\underline{H}$), 7.28-7.57 (m, 4H, arom. $\underline{H}$) ppm; GC-MS (EI, m/z, rel. int. (%)): 358, 360 ([M]$^+$, 4.1,1.9), 313, 315 (5.4, 2.1), 215 (8.4), 241, 243 (3.0, 1.4), 169 (23.6), 143, 145 (13.7, 6.1), 125 (11.8), 103 (100), 75 (66).

A suspension of **4** (2.5 g, 7.0 mmol) in 0.5N HCl (25 mL) was heated under reflux for 45 min. The cooled reaction mixture was extracted with CH_2Cl_2 (3x25 mL) and the organic extracts were washed with water (2x10 mL) and brine. Drying of the organic phase and evaporation of the solvent *in vacuo* gave **3b** (1.78 g, 90%) as an orange oil, which was almost pure. Purification by chromatography (florisil, diethyl ether) afforded pure **3b** (1.21 g, 61%). Because of its instability, **3b** was only characterized by ^{1}H-NMR and used immediately for further reactions. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.47, 1.51 (2s, 3H, C$\underline{H}_3$), 1.89-2.97 (m, 4H, C$\underline{H}_2$), 5.03 (m, 1H, C$\underline{H}$), 7.15-7.54 (m, 4H, arom. $\underline{H}$), 9.70, 9.76 (2s, 1H, $\underline{H}$C=O) ppm. Ratio of diastereomers 2:1.

3-[2-[4-(4-Chloro-phenylsulfanyl)-4-methyl-5-oxo-tetrahydrofuran-2-yl]-1-hydroxyethyl]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**5**)

A solution of tricyclic lactone **2** (835 mg, 4.80 mmol) in THF (10 mL) was added dropwise to a cooled (-78^{0}C) solution of LDA (4.80 mmol) in THF (15 mL) under N_2. The reaction mixture was stirred for 10 min. and then freshly prepared aldehyde **3b** (1.37 g, 4.80 mmol) in THF (5 mL) was added. Stirring was continued for 1 h at the same temperature. The reaction mixture was warmed up to 0^{0}C, stirred for 1 h and quenched with a saturated solution of NH_4Cl. Then THF was removed *in vacuo*. Extraction with ethyl acetate (3x20 mL) and washing of the combined organic extracts with water (2x) and brine, followed by drying provided crude product **5**. Purification by flash chromatography (SiO_2, eluens gradient hexane/ethyl acetate 3:1, 2:1 and then 3:2) gave seven fractions with different R_f-values, ranging from 0.17-0.062 (eluens hexane/ethyl acetate 2:1), which all showed satisfactory ^{1}H-NMR data. Total yield of all collected, pure fractions 1.46 g, 66%. From the fast moving fraction (R_f 0.17, hexane/ethyl acetate 2:1) pure crystals (106 mg) could be obtained by crystallization from hexane/ethyl acetate. Mp 192-195^{0}C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.48 (s, 3H, C$\underline{H}_3$), 1.86 (dd, 1H, ^{2}J 13.9 Hz, J 9.0 Hz, C$\underline{H}_2$ D-ring), 2.00-2.11 (m, 2H, C$\underline{H}_2$), 2.29 (t, 1H, J 7.9 Hz, OC(O)C$\underline{H}$), 2.55 (dd, 1H, J 5.4 Hz, ^{2}J 13.9 Hz, C$\underline{H}_2$ D-ring), 2.92 (d, 1H, ^{2}J 16.4 Hz, C$\underline{H}_2$ B-ring), 3.17 (ddd, 1H, J 7.9, 7.7, 7.6 Hz, C$\underline{H}$ BC-ring), 3.33 (dd, 1H, J 7.6 Hz, ^{2}J 16.4 Hz, C$\underline{H}_2$ B-ring), 3.66 (s, 1H, exchanges in the presence of D_2O, O$\underline{H}$), 4.18 (m, 1H, C$\underline{H}$OH), 4.96 (m, 1H, C$\underline{H}$ D-ring), 5.94 (d, 1H, J 7.7 Hz, PhC$\underline{H}$O), 7.27-7.49 (m, 8H, arom. $\underline{H}$) ppm; MS (CI, m/z, rel. int. (%)): 459, 461 ([M]$^+$+1, 0.23, 0.05), 441, 443 (0.35, 0.13), 284, 286 (1.74, 0.80), 174 (13.2), 144, 146 (36.6, 14.2), 129 (37.6), 41 (100); Analysis calcd for $C_{24}H_{23}ClO_5S$: C, 62.81; H, 5.05; S, 6.99. Found: C, 62.88; H, 4.98; S, 6.68.

3-[2-[4-(4-Chloro-phenylsulfanyl)-4-methyl-5-oxo-tetrahydrofuran-2-yl]-ethylidene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**Z-6**) *via the mesylate of* **5**

Typical procedure for the mesylation of **5**: To a solution of pure aldol **5** (R_f 0.17, eluens hexane/ethyl acetate 2:1) (50.1 mg, 0.11 mmol) in CH_2Cl_2 (3 mL) was added triethylamine (18.2 μL, 0.13 mmol) and then mesyl chloride (10.1 μL, 0.13 mmol) at 0^{0}C. The solution was stirred for 18 h before quenching with a saturated solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (2x10 mL) and the combined organic layers were washed with water (2x5 mL). Drying and evaporation of the solvent *in vacuo* afforded 46.0 mg (78%) of the desired mesylate, which was sufficiently pure for the next elimination reaction. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.41 (s, 3H, C$\underline{H}_3$), 1.81-3.48 (m, 7H), 3.08 (s, 3H, C$\underline{H}_3$ mesyl), 4.69 (m, 1H, C$\underline{H}$ D-ring), 5.22 (dt, 1H, J 3.3, 9.2 Hz, C$\underline{H}$OMs), 5.86 (d, 1H, J 7.6 Hz, PhC$\underline{H}$O), 7.34 (m, 8H, arom. $\underline{H}$) ppm; MS (CI, m/z,

rel. int. (%)): 536, 538 ([M]$^+$, 0.51, 0.21), 440, 442 (2.38, 1.28), 297 (1.16), 144, 146 (19.8, 10.2), 97 (8.15), 41 (100).
The crude mesylate (46.0 mg, 0.09 mmol) was treated with DBU (16.7 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at 0^0C. After 2h stirring at the same temperature the solution was quenched with a saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2 (2x10 mL). The combined organic layers were washed with water (2x5 mL) and dried. Removal of the solvent *in vacuo* gave almost pure product *Z*-**6**, which was purified by chromatography (SiO_2, hexane/ethyl acetate 2:1) to give 35 mg, 72% (based on starting aldol **5**) *Z*-**6** as a white solid. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.34 (s, 3H, C$\underline{H}_3$), 1.92 (dd, 1H, J 10.1 Hz, ^{2}J 14.1 Hz, C$\underline{H}_2$ D-ring), 2.38 (dd, 1H, J 5.5 Hz, ^{2}J 14.1 Hz, C$\underline{H}_2$ D-ring), 2.53-3.57 (m, 4H, 2C$\underline{H}_2$), 3.78 (m, 1H, C$\underline{H}$ BC-ring), 4.50 (m, 1H, C$\underline{H}$ D-ring), 5.86 (d, 1H, J 7.6 Hz, PhC$\underline{H}$O), 6.36 (ddd, 1H, J 6.9, 8.3 Hz, ^{4}J 2.3 Hz, =C$\underline{H}$), 7.19 (m, 8H, arom. $\underline{H}$) ppm; MS (EI, m/z, rel. int. (%)): 440, 442 ([M]$^+$, 65.6, 26.9), 296 (7.2), 279 (35.2), 225 (34.6), 143, 145 (92.0, 20.7), 109 (43.3), 41 (100); HRMS/EI: m/z calcd for $C_{24}H_{21}{}^{35}ClO_4S$ 440.0849. Found: 440.0849.

3-[2-[4-(4-Chloro-phenylsulfanyl)-4-methyl-5-oxo-tetrahydrofuran-2-yl]-ethylidene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (*E*-**6**)
Typical procedure starting from pure aldol **5** (R_f 0.17, eluens hexane/ethyl acetate 2:1): To a solution of aldol **5** (30.7 mg, 0.067 mmol) was added 29.9 mg, 0.10 mmol 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (95%, purchased from Aldrich), followed by triethylamine (20.3 mg, 0.20 mmol) at room temperature under N_2. The reaction mixture was stirred for 4h, quenched with a saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2 (2x10 mL). The combined organic layers were washed with water (2x5 mL) and dried. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 3:1) gave two geometrical isomers *Z*-**6** and *E*-**6**.
E-**6**. Yield 19.0 mg, 64% as a white solid. R_f 0.44 (eluens hexane/ethyl acetate 1:1). ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.44 (s, 3H, C$\underline{H}_3$), 2.04 (dd, 1H, J 10.2 Hz, ^{2}J 13.8 Hz, C$\underline{H}_2$ D-ring), 2.50 (dd, 1H, J 5.3 Hz, ^{2}J 13.8 Hz, C$\underline{H}_2$ D-ring), 2.57-3.66 (m, 4H, 2C$\underline{H}_2$), 3.85 (m, 1H, C$\underline{H}$ BC-ring), 4.70 (m, 1H, C$\underline{H}$ D-ring), 5.87 (d, 1H, J 7.5 Hz, PhC$\underline{H}$O), 6.68 (dt, 1H, J 7.5, ^{4}J 2.5 Hz, =C$\underline{H}$), 7.30 (m, 8H, arom. $\underline{H}$) ppm; MS (EI, m/z, rel. int. (%)): 440, 442 ([M]$^+$, 77.8, 32.1), 296 (19.0), 279 (10.7), 225 (42.5), 144, 146 (100, 38.2), 109 (53.6), 41 (65.8); HRMS/EI: m/z calcd for $C_{24}H_{21}{}^{35}ClO_4S$ 440.0849. Found: 440.0845.
Z-**6**. Yield 9.5 mg, 32% as a white solid. R_f 0.56 (eluens hexane/ethyl acetate 1:1). ^{1}H-NMR and mass data were the same as described for the compound obtained by mesylation and elimination (*vide supra*).

E-3-[2-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)ethylidene]-3,3a,4,8b-tetrahydroindeno-[1,2b]furan-2 -one (**1**)
Prepared similarly as described previously[14] starting from *Z*-**6** (150 mg, 0.34 mmol) as a mixture of diastereomers. Purification by chromatography (SiO_2, hexane/ethyl acetate 2:1) afforded **1** (95mg, 94%) as white solid in a diastereomeric ratio of 1:1 (calculated from ^{1}H-NMR analysis). An analytical sample was obtained by crystallization from hexane/ethyl acetate. R_f 0.26 (eluens hexane/ethyl acetate 1:1). ^{1}H-NMR and mass data were in complete agreement with those reported previously. Analysis calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.69; H, 5.52.

Z-3-[2-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)-ethylidene]-3,3a,4,8b-tetrahydroindeno-[1,2-b]furan-2 -one (**7**)

Prepared according to the procedure for the synthesis of **1** starting from **Z-7** (130 mg, 0.30 mmol) as a mixture of diastereomers. Yield after chromatography (SiO_2, hexane/ethyl acetate 2:1) 79 mg, 90% as a white solid. Diastereomeric ratio 1:1 as calculated from ^{1}H-NMR analysis. An analytical sample was obtained by crystallization from hexane/ethyl acetate. R_f 0.43 (eluens hexane/ethyl acetate 1:1). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.75, 1.93 (2s, 3H, C$\underline{H}_3$), 2.89-3.03 (m, 2H, =C-C$\underline{H}_2$ & C$\underline{H}_2$ B-ring), 3.34 (m, 1H, =C-C$\underline{H}_2$), 3.46-3.54 (m, 1H, C$\underline{H}_2$ B-ring), 3.84 (m, 1H, C$\underline{H}$ BC-ring), 4.98, 5.02 (2m, 1H, =C-C$\underline{H}$ D-ring), 5.93 (d, 1H, J 7.7 Hz, PhC$\underline{H}$O), 6.35, 6.37 (2dt, 1H, J 7.4Hz, ^{4}J 2.3Hz, =C$\underline{H}$), 6.98, 7.05 (2m, 1H, =C$\underline{H}$ D-ring), 7.24-7.51 (m, 4H, arom. $\underline{H}$) ppm; MS (CI, m/z, rel. int. (%)): 297 ([M+1]$^+$, 5.6), 199 ([$C_{13}H_{11}O_2$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 70.3); Analysis calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.79; H, 5.37.

Biological activity

Seeds

Seeds of *Striga hermonthica* (from *Sorghum bicolor* (L.) Moench) and *Orobanche crenata* (from *Vicia faba* L.) were harvested in Sudan in 1987 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of test solutions

A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a. and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0.1, and 0.01 mg/L test compound and 0.1, 0.01, and 0.001% (v/v) acetone, respectively.

Bioassays

For surface sterilization seeds of *Striga hermonthica* and *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30-70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20^{0}C for *Orobanche* seeds and at 30^{0}C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.1, 0.01 and 0.001% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (concentrations of 1, 0.1 and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks per treatment.

For full details of the bioassay, see Mangnus *et al.*[24]

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Chapter 11*

Synthesis and Biological Evaluation of Strigol Analogs Modified in the Enol Ether Part

Abstract: Several analogs of strigol, which is a germination stimulant for seeds of the parasitic weeds *Striga* and *Orobanche*, have been prepared. Structural modifications were introduced in the vinyl ether part and include i. analogs containing an endocyclic vinyl ether double bond, using tetronic acids as precursors; ii. analogs containing a methyl substituent on the vinyl ether double bond; iii. geometrical isomers of the vinyl ether double bond. During coupling reactions to give compounds belonging to the last-mentioned class undesired C-alkylation occurred, which could be minimized by choosing the appropriate reaction conditions. Bioassays revealed that the analogs prepared exhibit considerable activity in the stimulation of seed germination of *Striga hermonthica* and *Orobanche crenata*.

11.1 Introduction

The strigolactones[1] (+)-strigol,[2-4] sorgolactone[5] and alectrol[6] (fig 1) have been the subject of several structure-activity studies with respect to their high potency in the stimulation of germination of seeds of the parasitic weeds *Striga* and *Orobanche*. These studies revealed that the bioactiphore resides in the CD-part of the molecule.[7-10]

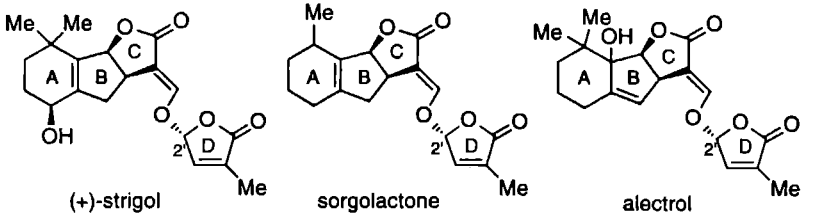

Figure 1

One of the most potent synthetic strigol analogs is GR24 (**4a**),[11,12] whose half-maximal activity is at 10^{-9}M for seeds of *Striga hermonthica* (Del.) Benth. Recently, a tentative molecular

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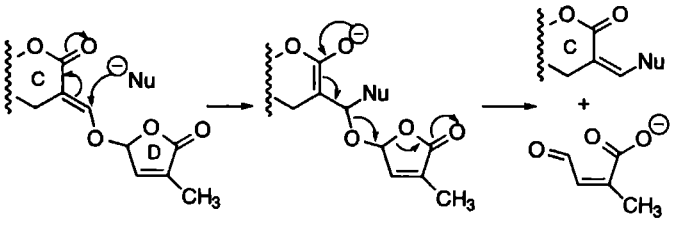
mechanism, which accounts for the germination stimulatory activity of strigol and its (synthetic) analogs has been proposed (scheme 1).[13]

Scheme 1



The enol ether unit plays a crucial role in this mechansim, as it enables the D-ring to eliminate as shown. Reduction of this moiety[13] or replacing the oxygen atom by a methylene function[14] results in complete loss of bioactivity.

The aim of the work described in this chapter is to study the influence of structural modifications in the enol ether part on the induction of germination of seeds of *Striga hermonthica* and *Orobanche crenata* Forsk., whereby its inherent reactivity (scheme 1) is retained. These modifications can be divided into three classes (fig. 2): i. Analogs possessing an endocyclic enol ether double bond instead of an exocyclic double bond, *viz.* as in structures **1a-c**. ii. Methyl substitution on the enol ether double bond of the strigol analogs **2a-5a**[9,10] resulting in analogs **2b-5b**. iii. Geometric isomers of the enol ether double bond as in **6**.

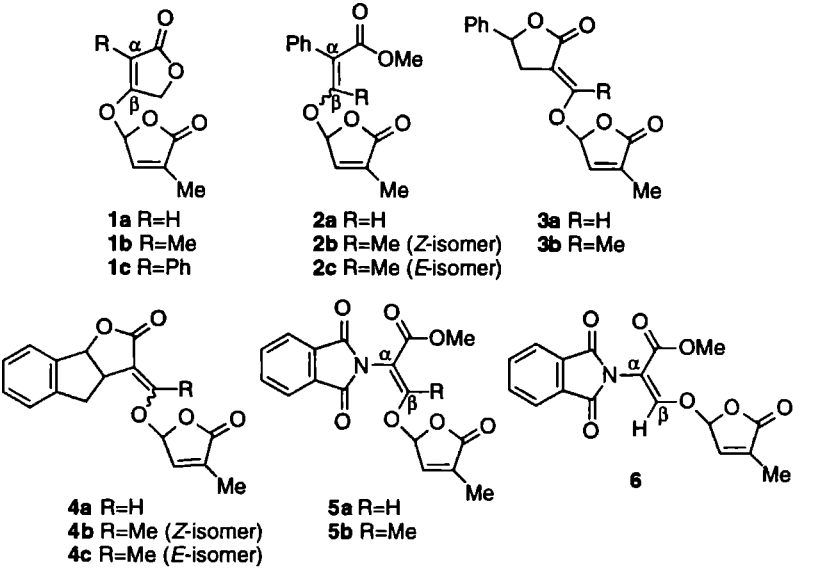


Figure 2

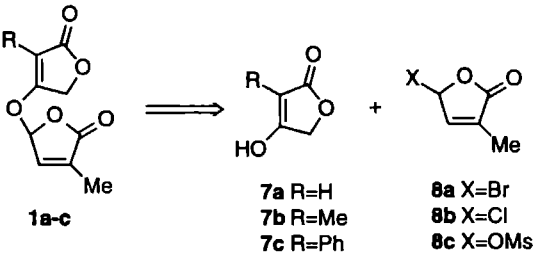
11.2 Results and discussion

Synthesis

Tetronic acid derived ABC-analogs

Retrosynthetic analysis of ABC strigol analogs **1a-c** suggests a coupling reaction of an appropriate β-tetronic acid **7** with 2(5H)-furanones **8**[12,15] as depicted in scheme 2.

Scheme 2



The chemistry of β-tetronic acids (4-hydroxy-2(5H)-furanones, $pK_a \approx 2$-4) has been reviewed.[16,17] Tetronic acids **7a-c** were obtained by standard procedures. Unsubstituted **7a** is commercially available, **7b** was prepared in a one pot synthesis by bromination of ethyl 2-methylacetoacetate to give the 2-bromo derivative,[18] which rearranged in the presence of hydrobromic acid to the corresponding 4-bromo derivative and on subsequent heating gave 2-methyl tetronic acid **7b**.[19] 3-Phenyl derivative **7c** was prepared via an internal Claisen condensation of methoxycarbonylmethyl phenylacetate.[20] Coupling of **7a-c** with **8a** using potassium *tert.* butoxide as the base in DMF, smoothly gave **1a-c**, which could readily be purified by crystallization without chromatography. The formation of O-alkylated compounds **1a-c** was established by ^{13}C-NMR and ^{1}H-NMR analysis. No indication of C-alkylation was observed.

Methyl substituted analogs 2b-5b

The preparation of **2b-5b** was expected to proceed in a similar manner as described for the synthesis of the desmethyl analogs **2a-5a**[10,15,21] from the precursors **9-12** (fig. 3) and 5-bromo-2(5H)-furanone **8a**.

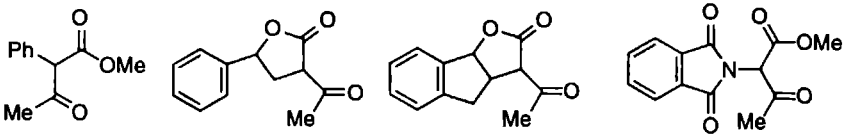
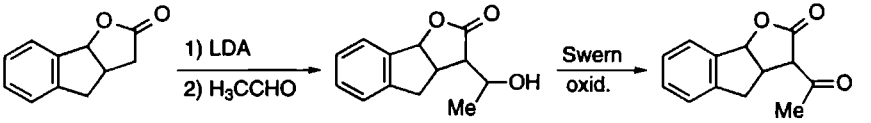


Figure 3

β-Keto ester **9** was prepared by condensation of methyl phenylacetate and methyl acetate following a modified literature procedure,[22] using KO^{t}Bu as the base in THF. It was essential to keep the reaction mixture at -78^{0}C to avoid complete self-condensation of methyl phenylacetate. Pure **9** was obtained in a moderate yield (39%) after distillation. The potassium enolate of β-keto lactone **10**[23]was prepared *in situ* in an analogous manner starting from commercially available γ-phenyl γ-butyrolactone, followed by quenching with **8a** (*vide infra*). Precursors **11** and **12** could not be obtained in satisfactory yields by direct condensation of tricyclic lactone **13** or methyl *N*-phthaloyl glycinate, respectively, with methyl acetate and therefore an alternative route was devised. Compound **11** was synthesized in two steps by condensation with acetaldehyde, followed by Swern oxidation (scheme 3).[24]

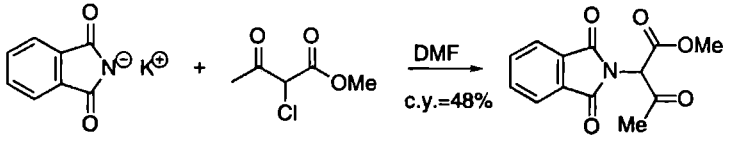
Scheme 3



13 **14** c.y.=93% **11** c.y.=73%

Compound **10** can also be prepared in good yields using this methodology. For the synthesis of **12** a different route had to be followed, *viz.* Gabriel condensation[25] of potassium phthalide with methyl α-chloroacetoacetate,[26] which gave **12** in a moderate yield (scheme 4). Purification of **12** was complicated by the presence of small amounts of phthalimide, which could only be removed by repeated crystallization.

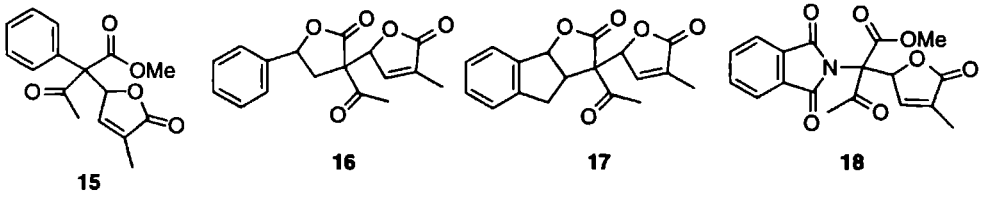
Scheme 4



12

*Coupling of **9-12** with **8a***

Compounds **9-12** were subjected to standard coupling conditions (KO^{t}Bu, DMF) with 5-bromo-2(5H)-furanone **8a** (*vide supra*). However, under these conditions almost exclusive formation of C-alkylated products **15-18** (fig. 4) as mixtures of diastereomers was observed (substrate **12** is an exception). Relevant data are collected in Table 1 (entries 1-4).



15 16 17 18

Figure 4

The structure of compounds **15-18** was primarily established by ^{13}C-NMR analysis and based on the signals between 195.2 and 202.5 ppm, which are characteristic for a ketone function.

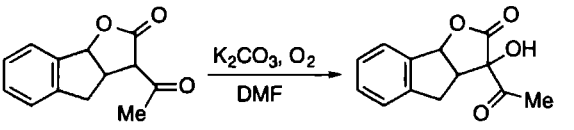
Table 1. Results of coupling reactions of **9-12** and **8a**

entry	precursor	base	solvent	ratio O:C[a]	ratio of diast.[b]	yield (%)[c]
1	**12**	KO^{t}Bu	DMF	2:3	6:1	67
2	**10**	KO^{t}Bu	DMF	C-alk.	8:2:1:0.2	34[e]
3	**11**	KO^{t}Bu	DMF	C-alk.	2:1	40
4	**9**	KO^{t}Bu	DMF	C-alk.	1:1	65
5	**9**	KO^{t}Bu[d]	THF	3:7	1:1	61
6	**9**	KO^{t}Bu[d]	toluene	7:3	1:1	69
7	**9**	DCA	DMF	C-alk.	1:1	30[f]
8	**9**	Ag_2O	CH_3CN	3:4	2:1	50
9	**12**	KO^{t}Bu[d]	THF			n.r.[g]
10	**12**	KO^{t}Bu[d]	toluene			n.r.[g]
11	**11**	KO^{t}Bu[d]	toluene	1:2	3:1	55

[a] Ratio of O-alkylated products **2b-5b** and the corresponding C-alkylated products **15-18** as determined by ^{1}H-NMR analysis of the crude reaction mixtures
[b] Diastereomeric ratio of the C-alkylated products **15-18**
[c] Combined yield of C- and O-alkylated products
[d] Addition of 0.5 equiv 18-crown-6
[e] Overall yield obtained after *in situ* acetylation of γ-phenyl γ-butyrolactone, followed by quenching with **8a**
[f] Ratio **15** **9** in crude reaction mixture
[g] No reaction

Further experiments showed that the reaction conditions are crucial for the product formation. It is important to carry out the reactions in an inert atmosphere. When compound **11** was subjected to the coupling conditions (KO^{t}Bu, DMF, alkylating agent) without exclusion of oxygen, oxidation to α-hydroxy compound **19** took place. The same reaction occurred upon treatment of **11** with potassium carbonate in DMF in the presence of oxygen, furnishing **19** as one single diastereomer according to GC and ^{1}H- and ^{13}C-NMR analysis (scheme 5). This reaction did not take place in an inert atmosphere. A similar type of reaction was observed on treatment of **10** with base in DMF in an oxygen atmosphere (data not shown).

Scheme 5



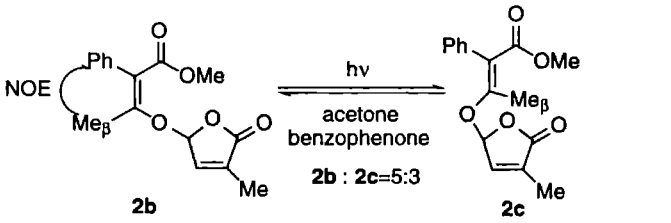
11 **19**, c y =34%
1 diastereomer

To further explore the influence of the reaction conditions on the product distribution, **9** was selected as a model substrate (entries 4-8, Table 1) Decreasing the polarity of the solvent, *viz* DMF>THF>toluene, resulted in a substantial increase of the O C ratio (entries 4-6, Table 1) In the two last-mentioned solvents the addition of 18-crown-6 was essential for a successful reaction as it enhanced the solubility of the potassium enolate considerably These results are in contrast with the expectations [27] Increasing the polarity of an aprotic solvent should decrease the S_N2-character of the transition state, which makes attack of the more electronegative oxygen atom of the ambident nucleophile more likely as is indicated by the HSAB-principle [28] Also the use of the bulky dicyclohexylammonium enolate (entry 7, Table 1) did not result in significant O-alkylation Apparently, solvatation of this bulky cation is not very effective Instead, it undergoes strong complexation with the enolate, and as a consequence attack of the less electronegative atom is favored The use of Ag^+ as the cation was considered, since it specifically helps in removing the leaving group This makes the transition state more S_N1-like, which indeed was reflected in the product distribution (entry 8, Table 1) The results collected in Table 1 apparently cannot be explained solely by electronic factors and steric factors should be considered as well S_N2-Reactivity is usually associated with steric effects,[29] which implies that attack of the enolate oxygen in an S_N2-like transition state is favored over attack by the highly branched α-carbon atom of enolates **9-12** This may explain the relatively high O C ratio when the reaction was carried out in an apolar solvent (entry 6, Table 1) It was attempted to increase the O C ratio further by using harder electrophiles, *e g* chloro furanone **8b** and mesyloxy furanone **8c** Surprisingly, **9** did not react under these conditions When the reactions were carried out in DMF or toluene in the presence of 18-crown-6, **9** was recovered almost quantitatively Attempts to enhance O-alkylation of **12** with bromo furanone **8a** in THF and toluene (entries 9 and 10, Table 1) failed, which is most likely due to the poor solubility of its potassium enolate even in the presence of 18-crown-6 Application of the optimal conditions for O-alkylation, found for **9**, gave access to the GR24-analogs **4b**, although C-alkylation was the predominant reaction (entry 11, Table 1) These results indicate that also the nature of the β-keto ester plays a determining role in the ratio of O- and C-alkylation Under similar reaction conditions the O C ratio increases in the order **11<9<12** The relatively strong tendency of **12** to undergo O-alkylation agrees with its presence in the enol form in $CDCl_3$ The relative stabilities of the enol and keto tautomers may reflect the relative activation energies leading to O- and C-alkylation

Assignment of E/Z-geometry of 2b, 4b and 5b

Compounds **2b**, **4b** and **5b** were subjected to 2D-NOESY NMR analysis in order to determine unambigiously the correct geometry at the vinyl ether double bond. However, for **5b** this analysis did not provide sufficient information to assign its configuration and therefore an X-ray diffraction analysis was undertaken. The crystal structure[30] clearly showed the *Z*-configuration as is depicted in fig. 2. Analysis of the NOESY-data of **2b** reveals the *Z*-configuration, as was deduced from the observed NOE cross-peak between the β-methyl and phenyl groups (scheme 6).

Scheme 6



In order to obtain additional support for this assignment, **2b** was photoisomerized in acetone in the presence of benzophenone, to give a mixture of **2b** and **2c** (scheme 6). The β-methyl group in **2c** displayed an 0.7 ppm downfield shift in the ^{1}H-NMR spectrum as compared to the corresponding methyl group in **2b** and did not show a NOE contact with the phenyl group. This NOESY-experiment was performed using a mixture of **2b** and **2c**, because it was not possible to separate these compounds by chromatography. A correct interpretation could nevertheless readily be achieved, since the corresponding relevant proton signals of **2b** and **2c** could clearly be distinguished. Similarly, based on the observed relevant NOE contacts as is depicted in fig. 5, the structure of **4b** has the *Z*-configuration as shown (fig. 5).

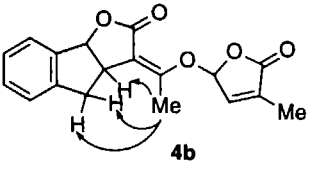


Figure 5

Thus, combination of the NOESY-data and molecular mechanics calculations of the relevant intramolecular atom distances strongly disfavors the possibility of the alternative isomers, *viz.* **2c** and **4c**, as the products of the coupling reactions (Table 1). The stereochemical

outcome of the reactions to give the *Z*-isomers **2b** and **4 b** is quite unexpected, as the corresponding desmethyl derivatives **2a** and **4a** are exclusively obtained in an *E*-selective fashion.[10,15] This behavior cannot be explained as yet.

Photoisomerization of 5a

Isomerization of the enol ether double bond of compound **5a** was achieved by irradiation with UV-light in toluene in the presence of benzophenone as sensitizer. Similar conditions, using another strigol analog, were reported previously,[31] but no data of the effect on the bioactivity were given. In our hands, a 1:1 mixture of **5a** and **6** was formed. Evidence for the isomerization was obtained by the upfield shift (0.8 ppm) of the β-proton of **6** in the ^{1}H-NMR spectrum as compared to the same proton of **5a**.

Biological evaluation

The germination stimulatory activity of phthalimidoglycine derived strigol analogs **5a,b** and **6**, and tetronic acid derivatives **1a-c** was assayed using seeds of *Striga hermonthica* and *Orobanche crenata*. In each bioassay, GR24 **4a** at an optimal concentration (0.01 mg/L for *Striga hermonthica* and 1 mg/L for *Orobanche crenata*) was included as a positive control. This enables a comparison between results obtained in different test series. The reference test is important, since the respons of seeds of parasitic weeds, in particular of *Striga hermonthica*, varies considerably from test to test. The germination percentages are collected in Tables 2a and 2b.

Table 2a. Germination stimulatory activity of strigol analogs **1**, **5**, and **6** toward seeds of *Orobanche crenata*[a]

		% germination ± S.E.			
entry	compound	2 mg/L	1 mg/L	0.01 mg/L	**4a** 1 mg/L[b]
1	**5a**	58.3±1.3	42.6±1.8	0.0±0.0[c]	66.1±1.8
2	**5b**		43.2±3.2	0.5±0.3[c]	74.9±1.0
3	**6**	43.2±3.5	23.1±2.4	0.0±0.0[c]	66.1±1.8
4	**1a**		47.4±4.7	0.5±0.3[c]	73.7±1.2
5	**1b**		34.6±2.2	1.6±0.8[c]	68.2±1.3
6	**1c**		29.3±0.3	0.8±0.4[c]	68.2±1.3

[a] Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 500 seeds, distributed over 9 discs, of one representative experiment.

[b] Mean germination percentages ± S.E. obtained by treatment of the seeds with GR24 **4a** (1 mg/L) in the same bioassay

[c] Values are not significantly different from germination percentages obtained in the control (without stimulant)

Table 2b. Germination stimulatory activity of strigol analogs **1**, **5**, and **6** toward seeds of *Striga hermonthica*[a]

		% germination ± S.E.		
entry	compound	1 mg/L	0.01 mg/L	**4a** 0.01 mg/L[b]
1	**5a**	37.3±5.0	4.6±0.9[c]	45.0±5.4
2	**5b**	36.8±2.0	34.1±3.2	47.5±2.2
3	**6**	44.1±1.6	3.1±0.9[c]	78.3±3.0
4	**1a**	29.6±2.4	8.5±0.7[c]	47.5±2.2
5	**1b**	45.9±5.0	14.5±2.2	51.7±4.1
6	**1c**	54.9±5.1	17.9±3.6	78.3±3.0

[a] Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions Germination percentages given are means ± S.E. by counting *ca.* 550 seeds, distributed over 12 discs, of one representative experiment

[b] Mean germination percentages ± S.E. obtained by treatment of the seeds with GR24 **4a** (0.01 mg/L) in the same bioassay

[c] Values are not significantly different from germination percentages obtained in the control (without stimulant)

The activity of both diastereomers of GR24 analogs **4b** was assessed and compared with that of GR24 **4a** at the concentrations 1 mg/L and 0.1 mg/L, using seeds of *Orobanche crenata*, the results of which are collated in Table 2c.

Table 2c. Germination stimulatory activity of GR24 (**4a**) and **4b** toward seeds of *Orobanche crenata*[a]

		% germination ± S.E.	
entry	compound	1 mg/L	0.1 mg/L
1	**4a**[b]	59.0±6.7	43.6±2.9
2	**4b**[c]	23.1±6.8	5.7±5.3
3	**4b**[d]	14.5±4.1	3.3±0.4
4	aq. control	0.6±0.3	

a) Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S E by counting *ca.* 400 seeds, distributed over 9 discs, of one representative experiment.
b) Tested as an 1 1 mixture of diastereomers
c) Fast moving diastereomer as indicated by its chromatographic behavior on TLC
d) Slow moving diastereomer as indicated by its chromatographic behavior on TLC

Compounds **1**, **5** and **6** stimulate germination of both types of seeds at 1 mg/L. These compounds were virtually inactive with respect to seed germination of *Orobanche crenata* at a concentration of 0.01 mg/L. It should be noted that in this assay also GR24 **4a** was only moderately active at this concentration. A modest stimulatory activity of tetronic acid derivatives **1b-c** at 0.01 mg/L (*Striga hermonthica*) was observed (entries 5-6, Table 2b), whereas methyl substituted analog **5b** stimulates germination considerably at this concentration (entry 2, Table 2b). Comparison of the germination percentages of compounds **5a-b** at the lower concentration

(entries 1-2, Table 2b) indicates that a methyl substituent at the enol ether moiety is beneficial for the bioactivity. None of the isomeric C-alkylated analogs **15**, **16** and **18** exert any stimulatory effect (data not shown). The presented data indicate that there is a considerable degree of structural freedom at the enol ether function without affecting the bioactivity to a large extent. These results can be summarized as follows: i. The presence of a small substituent at C_β has no negative effect on the bioactivity (entries 2, 4-6, Tables 2a-b). ii. The E/Z-geometry of the enol ether double bond in **5a** is not essential to retain stimulatory activity (*cf.* entries 1 and 3, Tables 2a-2b). Thus, interchanging the phthalimido- and methyl carboxylate-group leads to retention of bioactivity. This means that these functions are bioisosteric.[32] iii. On the other hand, double bond isomerization including the relatively bulky ABC-part such as in **4b** (Table 2c) leads to a substantial loss of bioactivity.

The activity of tetronic acid analog **1c** is comparable to that of the closely related compound **2a**.[10] Tetronic acid analog **1a** only contains the minimal essential structural features,[13] *viz.* the α,β-unsaturated enol ether moiety, which allows an addition/elimination mechanism (scheme 1), and the 3-methyl-2(5H)-furanone fragment. Analogs **1**, **2b-5b** and **6** were designed in such a manner that the essence of the molecular mechanism (scheme 1) is retained. These results are in full accord with the finding of complete loss of germination activity when the connecting unit was modified, *viz.* reduction of the olefinic bond[13] or replacement of the oxygen atom by a methylene function (chapter 10),[14] and thus the results described above provide a further substantiation of the proposed molecular mechanism. It may thus be concluded that, in order to retain germination stimulatory activity, the presence of the enol ether moiety is more important than its spatial arrangement.

11.3 Experimental section

Synthesis

General remarks

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 100 (100 MHz) or a Bruker AM-400 (400 MHz) spectrometer (Me_4Si as internal standard). All coupling constants are given as ^{3}J in Hz, unless indicated otherwise. IR spectra were measured on a Perkin Elmer 298 spectrophotometer. For mass spectra a double focussing VG7070E mass spectrometer was used. Melting points were determined using a Reichert thermopan microscope. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.

Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH_2. Ethyl acetate was distilled from potassium carbonate. Tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0.25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray. Flash-chromatography was carried out at a pressure of *ca.* 1.5 bar, using Merck Kieselgel 60H. Column chromatography at

atmospheric pressure was carried out, using Merck Kieselgel 60. Irradiations were carried out using a Hanau TQ150 high pressure mercury vapour lamp (150 W), equipped with a pyrex filter. 3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one **13**[15] and 5-bromo-3-methyl-2(5H)-furanone **8a**[15] were prepared following published methods.

4-Hydroxy-3-phenyl-2(5H)-furanone 7c
This compound was prepared similarly as described by Campbell *et al.*,[20] starting from methoxycarbonylmethyl phenylacetate (0.1 mol) and potassium *tert*-butoxide (0.11 mol) in *tert*-butyl alcohol (200 mL). After work-up and recrystallization from ethanol analytically pure **7c** (6.8 g, 50%) was obtained, exhibiting identical spectroscopic data as reported previously.[20] The starting methoxycarbonylmethyl phenylacetate was obtained as follows: To a cooled (0^0C) solution of phenylacetic acid (13.6 g, 100 mmol) and methyl hydroxyacetate (9.00 g, 100 mmol) in THF (150 mL) were added DCC (21 g, 0.10 mol) and a catalytic amount of DMAP. The mixture was allowed to warm to room temperature and stirred overnight at reflux temperature. THF was removed *in vacuo* and to the residue was added di*iso*propyl ether (150 mL). The precipitate (DCU) was removed by filtration. The filtrate was concentrated *in vacuo* to give methoxycarbonylmethyl phenylacetate as an oil in quantitative yield, which was sufficiently pure for use in the next reaction.
^{1}H-NMR ($CDCl_3$, 100 MHz): δ 3.74 (s, 5H, $OC\underline{H}_3$ + $PhC\underline{H}_2$), 4.63 (s, 2H, $OC\underline{H}_2$), 7.31 (s, 5H, arom. H) ppm.

Coupling of 4-hydroxy-2(5H)-furanones 7a-c with 5-bromo-3-methyl-2(5H)-furanone 8a (general procedure)
To a solution of 4-hydroxy-2(5H)-furanone **7a-c** (10 mmol) in DMF (40 mL) was added potassium *tert*-butoxide (11 mmol) under a nitrogen atmosphere. The mixture was cooled to -60^0C and a solution of **8a** (11 mmol) in DMF (10 mL) was added with stirring. Stirring was continued for 18 h at room temperature. Then, DMF was removed *in vacuo* and the residue was dissolved in a mixture of water and ethyl acetate. The aqueous layer was extracted with ethyl acetate (2x) and the combined organic extracts were washed with satd. NH_4Cl and water, dried ($MgSO_4$) and concentrated *in vacuo* to give **1a-c** as solids.

3-Methyl-5-(5-oxo-2,5-dihydro-furan-3-yloxy)-2(5H)-furanone (**1a**)
Following the general procedure 4-hydroxy-2(5H)-furanone **7a** (1.0 g, 10 mmol) gave after work-up 1.5 g (76%) as a yellow solid. An analytically pure sample was obtained by repeated recrystallization from ethanol to give **1a** as white crystals. Mp 128^0C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.05 (m, 3H, $C\underline{H}_3$), 4.70 and 4.71 (2s, 2H, $C\underline{H}_2$), 5.50 (m, 1H, =C<u>H</u> C-ring), 6.27 (m, 1H, OC<u>H</u>O), 7.00 (m, 1H, =C<u>H</u> D-ring) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.6 (q, $\underline{C}H_3$), 67.5 (t, $\underline{C}H_2$), 93.3 (d, O<u>C</u>HO), 98.5 (d, =<u>C</u>H C-ring), 135.5 (s, =<u>C</u>-CH_3), 140.4 (d, =<u>C</u>H D-ring), 169.9 (s, =<u>C</u>O), 172.1 (s, <u>C</u>=O), 175.8 (s, <u>C</u>=O); MS (EI, m/z, rel. int. (%)): 197 ($[M+1]^+$, 3.1), 97 ($[C_5H_5O_2]^+$, 100); Analysis calcd for $C_9H_8O_5$: C, 55.12; H, 4.11. Found: C, 55.09; H, 4.09.

3-Methyl-5-(4-methyl-5-oxo-2,5-dihydro-furan-3-yloxy)-2(5H)-furanone (**1b**)
Following the general procedure 4-hydroxy-3-methyl-2(5H)-furanone **7b** (1.1 g, 10 mmol) gave after work-up 1.7 g (82%) as a yellow solid. An analytically pure sample was obtained by recrystallization from ethanol to give **1b** as white crystals. Mp 108-110^0C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.85 (m, 3H, $C\underline{H}_3$ C-ring), 2.04 (m, 3H, $C\underline{H}_3$ D-ring), 4.79 (m, 2H, $C\underline{H}_2$), 6.29 (m, 1H,

OCHO), 7.00 (m, 1H, =CH) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 6.8 (q, CH_3), 10.4 (q, CH_3), 65.8 (t, CH_2), 97.1 (d, OCHO), 103.3 (s, =C-CH_3 C-ring), 135.2 (s, =C-CH_3 D-ring), 140.9 (d, =CH), 168.0 (s, =CO), 169.9 (s, C=O), 173.8 (s, C=O) ppm; MS (EI, m/z, rel. int. (%)): 210 ($[M]^+$, 1.0), 97 ($[C_5H_5O_2]^+$, 100); Analysis calcd for $C_{10}H_{10}O_5$: C, 56.08; H, 4.71. Found: C, 56.70; H, 4.64.

3-Methyl-5-(4-phenyl-5-oxo-2,5-dihydro-furan-3-yloxy)-2(5H)-furanone (**1c**)
Following the general procedure starting from 4-hydroxy-3-phenyl-2(5H)-furanone **7c** (1.0 g, 5.7 mmol) gave after work-up 1.1 g (68%) as a yellow solid. An analytically pure sample was obtained by recrystallization from ethanol to give **1c** as white crystals. Mp 155-157°C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.04 (m, 3H, CH_3), 5.02 (AB, 2H, J_{AB} 17 Hz, CH_2), 6.26 (m, 1H, OCHO), 7.03 (m, 1H, =CH), 7.37 (m, 3H, arom. H), 7.82 (m, 2H, arom. H) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.3 (q, CH_3), 65.1 (t, CH_2), 97.3 (d, OCHO), 106.5 (s, =C-Ph), 127.8 (d, Ph), 128.1 (s, Ph), 128.3 (d, Ph), 128.4 (d, Ph), 135.5 (s, =C-CH_3), 140.9 (d, =CH), 168.6 (s, =CO), 169.8 (s, C=O), 171.4 (s, C=O) ppm; MS (EI, m/z, rel. int. (%)): 272 ($[M]^+$, 2.4), 176 ($[C_{10}H_8O_3]^+$, 9.4), 97 ($[C_5H_5O_2]^+$, 100); Analysis calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 66.21; H, 4.34.

Methyl 3-oxo-2-phenyl-butyrate (**9**)
By a modified literature procedure,[22] a mixture of methyl phenylacetate (30.1 g, 200 mmol) and methyl acetate (30.0 g, 405 mmol) was gradually added to a cooled (-78°C) solution of potassium *tert*-butoxide (24.7 g, 202 mmol) in a nitrogen atmosphere. The mixture was stirred for 2h at the same temperature and then allowed to warm to room temperature. Stirring was continued for 18h at room temperature and 1h under reflux. The mixture was cooled (0°C) and neutralized with acetic acid (1 eq.). The volatiles were removed *in vacuo* and the residue was dissolved in water and ethyl acetate. The aqueous phase was extracted with ethyl acetate (3x) and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Distillation under reduced pressure gave **9** (14.9 g, 39%) as a colorless oil, which solified on standing. The physical properties were in agreement with those reported previously.[22] ^{1}H-NMR ($CDCl_3$, 100 MHz), ratio keto:enol 2:1: δ 1.84 (s, 3H, CH_3 enol), 2.17 (s, 3H, CH_3 keto), 3.67 (s, 3H, OCH_3 enol), 3.74 (s, 3H, CH_3 keto), 4.72 (s, 1H, CH keto), 7.35 (m, 5H, arom. H keto and enol), 13.04 (br s, 1H, OH enol) ppm.

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-oxo-butanoate (**12**)
To a cooled (-30°C) solution of methyl 2-chloro-3-oxo-butanoate (6.03 g, 40.1 mmol) in DMF (30 mL) was gradually added potassium phthalimide (8.15 g, 44.1 mmol). The mixture was stirred for 1h at a temperature between -20°C and -30°C. Stirring was continued for 18h at room temperature. Insoluble salts were removed by filtration over hyflo. The filtrate was poured into ice/water and the pH was adjusted to 1-2 with 2N HCl. A precipitate gently settled, which was collected by filtration. Yield 6.3 g of a white solid, which consisted of **12** and phthalimide in a ratio 75:25 according to ^{1}H-NMR analysis. The residue was recrystallized from *n*-butyl acetate to give 1.32 g phthalimide as white crystals. The filtrate was concentrated *in vacuo* to give 5.0 g (48%) of a solid, which contained almost pure **12** together with a trace amount of phthalimide. Analytically pure **12** was obtained by recrystallization thrice from 2-propanol as pale yellow crystals. Mp 126-127°C; ^{1}H-NMR ($CDCl_3$, 100 MHz), ratio keto:enol 0:1: δ 1.95 (s, 3H, CH_3 enol), 3.71 (s, 3H, OCH_3), 7.87 (m, 4H, arom. H), 12.68 (br s, 1H, OH enol) ppm; IR (KBr): ν 3200 (broad, OH), 1722 (C=O, imide, ester), 1668 (C=C, enol) cm^{-1}; MS (EI, m/z, rel. int. (%)):

261 ([M]$^+$, 31.4), 219 ([$C_{11}H_9O_4N$]$^+$, 41.2), 132 ([$C_8H_4O_2$]$^+$, 100); Analysis calcd for $C_{13}H_{11}NO_5$: C, 59.77; N, 5.36; H, 4.24. Found: C, 59.83; N, 5.23; H, 4.29

3-(1-Hydroxyethyl)-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**14**)
A solution of tricyclic lactone **13** (2.61 g, 15.0 mmol) in THF (25 mL) was added dropwise to a cooled (-78^{0}C) solution of freshly prepared LDA (16.5 mmol) in THF (25 mL) under nitrogen. The reaction mixture was stirred for 15 min. at the same temperature and then warmed to -40^{0}C. Freshly distilled acetaldehyde (1.27 mL, 22.7 mmol) was added via a syringe and stirring was continued for 1 h at -40^{0}C. The reaction mixture was warmed to 0^{0}C and stirred for 1 h. Work-up was accomplished by quenching with 1N HCl (65 mL), removing THF *in vacuo*, followed by extraction with dichloro methane (3x). The combined organic layers were washed with water (2x) and dried ($MgSO_4$). Purification by chromatography (SiO_2, eluens hexane/ethyl acetate 2:1) afforded pure **14** (3.05 g, 93%) as a white solid, consisting of two diastereomers in a 1:1 ratio according to capillary GC and ^{1}H-NMR analysis. For characterization purposes one diastereomer was obtained in a pure form by stirring in di*iso*propyl ether, filtration and recrystallization the residue from hexane/ethyl acetate.
Diastereomer **14a**. Mp 117-120^{0}C; R_f 0.20 (hexane/ethyl acetate 2:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.36 (d, 3H, J 6.5 Hz, C$\underline{H}_3$), 2.10 (d, 1H, J 5.4 Hz, O$\underline{H}$), 2.42 (dd, 1H, J 7.6 Hz, J 3.2 Hz, O=CC$\underline{H}$), 2.92 (d, 1H, ^{2}J 16.2 Hz, C$\underline{H}_2$), 3.36 (dd, 1H, ^{2}J 16.2 Hz, J 8.0 Hz, C$\underline{H}_2$), 3.44 (m, 1H, $CH_2C\underline{H}$), 4.42 (m, 1H, C$\underline{H}$OH), 5.90 (d, 1H, J 7.7 Hz, PhC$\underline{H}$), 7.28-7.37 (m, 3H, arom. $\underline{H}$), 7.47 (d, 1H, J 7.3 Hz, arom. $\underline{H}$) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): (mixture of two diastereomers) δ 20.3, 20.9 (q, $\underline{C}H_3$), 37.0, 37.7 (t, $\underline{C}H_2$), 37.6, 40.6 (d, $CH_2\underline{C}H$), 52.6, 54.1 (d, C(O)$\underline{C}$H), 65.9, 68.0 (d, H$\underline{C}$OH), 86.0, 86.3 (d, Ph$\underline{C}$HO), 125.2, 125.3, 125.8, 125.9, 127.3, 127.4, 129.6, 129.7 (d, Ph), 138.5, 138.9, 141.4, 141.8 (s, Ph), 178.2, 178.8 (s, $\underline{C}$=O) ppm; IR (KBr): ν 3540 (broad, OH), 1750 (C=O, lactone) cm^{-1}; GC-MS (EI, m/z, rel. int. (%)): 218 ([M]$^+$, 75.7), 201 ([$C_{13}H_{13}O_2$]$^+$, 100), 128 ([$C_{10}H_8$]$^+$, 81.6), 115 ([C_9H_7]$^+$, 42.1); HRMS/EI: m/z calcd for $C_{13}H_{14}O_3$: 218.0943. Found 218.0944±0.0010; Analysis calcd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 70.65; H, 6.31
Diastereomer **14b** was obtained as a colorless oil, slightly contaminated with **14a** after stirring in di*iso*propyl ether and concentration *in vacuo*. R_f-value and mass data were the same as for **14a**.

3-Acetyl-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**11**)
To a stirred solution of distilled oxalyl chloride (1.92 g, 15.1 mmol) in dichloromethane (25 mL) was added DMSO (2.36 g, 30.3 mmol) at room temperature under nitrogen. The solution was cooled to -78^{0}C, followed by dropwise addition of a solution of **15** (3.00 g, 13.8 mmol) in dichloromethane (20 mL). Stirring was continued for 10 min at -78^{0}C and then a solution of triethylamine (6.96 g, 68.7 mmol) in dichloromethane was gradually added. After 5 min the reaction mixture was warmed to room temperature and quenched with 1N HCl (30 mL). Extraction with dichloromethane (2x), washing the combined organic extracts with water (1x) and drying ($MgSO_4$) provided **11** (2.86 g, 96%) as a yellowish oil, which was sufficiently pure for further use. Ratio keto:enol tautomers 3:2 according to ^{1}H-NMR analysis. R_f 0.30 (hexane/ethyl acetate 4:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.04 (d, 3H, C$\underline{H}_3$ enol), 2.40 (s, 3H, C$\underline{H}_3$ keto), 2.85 (dd, 1H, J 3.3 Hz, ^{2}J 16.8 Hz, C$\underline{H}_2$ keto), 3.02 (dd, 1H, J 3.4 Hz, ^{2}J 16.5 Hz, C$\underline{H}_2$ enol), 3.35 (dd, 1H, J 8.6 Hz, ^{2}J 16.8 Hz, C$\underline{H}_2$ keto), 3.49 (dd, 1H, J 8.7 Hz, ^{2}J 16.5 Hz, C$\underline{H}_2$ enol), 3.56 (d, 1H, J 6.1 Hz, C$\underline{H}$C(O)CH_3 keto), 3.84 (m, 1H, $CH_2C\underline{H}$ keto), 3.91 (m, 1H, $CH_2C\underline{H}$ enol), 5.91 (d, 1H, J 7.4 Hz, $\underline{H}$CO keto), 5.97 (d, 1H, J 8.2 Hz, $\underline{H}$CO enol), 7.26-7.47 (m, 4H, arom. $\underline{H}$), 11.21 (br s,

1H, OH) ppm; GC-MS (EI, m/z, rel. int. (%)): 216 ($[M]^+$, 11.3), 174 ($[C_{11}H_{10}O_2]^+$, 14.6), 129 ($[C_{10}H_9]^+$, 88.8), 115 ($[C_9H_7]^+$, 20.3), 43 ($[C_2H_3O]^+$, 100); HRMS/EI: m/z calcd for $C_{13}H_{12}O_3$: 216.0786. Found 216.0787±0.0010

3-Acetyl-3-hydroxy-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**19**)
To a stirred solution of acetyl lactone **11** (216 mg, 1.00 mmol) in DMF (10 mL) was added potassium carbonate (152 mg, 1.10 mmol) under atmospheric conditions with protection from moisture. The reaction mixture was stirred over a weekend at room temperature. DMF was removed *in vacuo*. The residue was dissolved in satd. NH_4Cl and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2x) and the combined organic extracts were washed with water (1x), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, hexane/ ethyl acetate 2:1) provided **19** (80 mg, 34%) as a white solid, which was according to capillary GC, ^{1}H- and ^{13}C-NMR analysis diastereomerically pure. Analytically pure **19** was obtained after recrystallization from hexane/ethyl acetate. Mp 152-155^{0}C; R_f 0.21 (hexane/ethyl acetate 2:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.89 (m, 3H, C$\underline{H}_3$), 2.91 (dd, 1H, ^{2}J 17.6 Hz, J 3.4 Hz, C$\underline{H}_2$), 3.27 (dd, 1H, ^{2}J 17.6 Hz, J 9.5 Hz, C$\underline{H}_2$), 3.52 (m, 1H, CH$_2$C$\underline{H}$), 4.40 (s, 1H, O$\underline{H}$), 6.13 (d, 1H, J 7.8 Hz, $\underline{H}$CO), 7.25 (d, 1H, J 7.1 Hz, arom. $\underline{H}$), 7.38 (m, 2H, arom. $\underline{H}$), 7.57 (d, 1H, J 7.1 Hz, arom. $\underline{H}$), ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 26.4 (q, $\underline{C}H_3$), 32.1 (t, $\underline{C}H_2$), 49.3 (d, CH$_2\underline{C}$H), 84.4 (s, H$\underline{C}$OH), 85.5 (d, H$\underline{C}$O), 124.7, 126.1, 127.9, 130.4 (4xd, Ph), 137.6, 142.3 (2xs, Ph), 174.2 (s, O$\underline{C}$=O), 204.1 (s, $\underline{C}$=O) ppm; IR (KBr): ν 3400 (broad, OH), 1750 (C=O, lactone), 1705 (C=O, ketone); GC-MS (EI, m/z, rel. int. (%)): 214 ($[M-H_2O]^+$, 18.0), 172 ($[C_{11}H_8O_2]^+$, 22.1), 115 ($[C_9H_7]^+$, 100), 43 ($[C_2H_3O]^+$, 92.2); Analysis calcd for $C_{13}H_{12}O_4$: C, 67.24, H, 5.21. Found, C, 66.98, H, 5.21.

Coupling of compounds ***9-12*** *with 5-bromo-3-methyl-2(5H)-furanone* ***8a*** *(general procedure)*
To a stirred solution of precursor **9-12** (2.50 mmol) in DMF (10 mL, procedure A) or toluene (10 mL, procedure B) was added potassium *tert*-butoxide (2.50 mmol) at room temperature under nitrogen, in procedure B followed by the addition of 18-crown-6 (1.25 mmol). The thus obtained solution was cooled to -65^{0}C (procedure A) or -78^{0}C (procedure B). A solution of **8a** (3.00 mmol) in DMF (3 mL, procedure A) or toluene (3 mL, procedure B) was gradually added. The reaction mixture was allowed to warm slowly to room temperature and stirring was continued for 15h. The solvent was removed *in vacuo* and the residue was dissolved in satd. NH_4Cl and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2x) and the combined organic extracts were washed with water (1x) and brine (1x), dried ($MgSO_4$) and concentrated *in vacuo*.

Methyl 2-(4-methyl-5-oxo-2,5-dihydro-furan-2-yl)-3-oxo-2-phenyl-butanoate (**15**)
Following procedure A, **9** (269 mg, 1.38 mmol) gave a crude product with a ratio of diasteromers of 1:1, according to ^{1}H-NMR analysis. Purification by flash chromatography (SiO_2, eluens gradient hexane/ethyl acetate 9:1, 6:1, 3:1) gave two separated diastereomers in a yield of 65%.
Diastereomer **15a**: Obtained as a white solid. Recrystallization from di*iso*propyl ether provided analytically pure **15a**. Mp 122-123^{0}C; R_f 0.21 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.59 (m, 3H, =CC$\underline{H}_3$), 2.23 (s, 3H, C$\underline{H}_3$), 3.90 (s, 3H, OC$\underline{H}_3$), 5.85 (m, 1H, $\underline{H}$CO), 6.94 (m, 1H, =C$\underline{H}$), 7.07-7.36 (m, 5H, arom. $\underline{H}$) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.2 (q, $\underline{C}H_3$), 27.7 (q, $\underline{C}H_3$), 53.4 (q, O$\underline{C}H_3$), 72.1 (s, $\underline{C}C_4$), 81.3 (d, H$\underline{C}$O), 128.5, 128.6, (2xd, Ph), 131.1 (s, Ph)*, 131.6 (s, =$\underline{C}$CH$_3$)*, 146.8 (d, =$\underline{C}$H), 166.0 (s, O$\underline{C}$=O), 173.2 (s, O$\underline{C}$=O), 202.0 (s, O=$\underline{C}$CH$_3$) ppm (*: signals may be interchanged); IR (KBr): ν 1758 (C=O, lactone), 1712 (C=O, ester,

acetyl) cm^{-1}, MS (EI, m/z, rel int (%)) 288 ($[M]^+$, 1 7), 246 ($[C_{14}H_{14}O_4]^+$, 99 1), 191 ($[C_{11}H_{11}O_3]^+$, 61 3), 187 ($[C_{12}H_{11}O_2]^+$, 100), 97 ($[C_5H_5O_2]^+$, 54 3), 43 ($[C_2H_3O]^+$, 70 9), Analysis calcd for $C_{16}H_{16}O_5$ C, 66 66, H, 5 59 Found C, 66 68, H, 5 61

Diastereomer **15b** Obtained as a colorless oil Purity according to capillary GC > 99% R_f 0 18 (hexane/ethyl acetate 3 1), ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 1 69 (m, 3H, =CC<u>H</u>$_3$), 2 25 (s, 3H, C<u>H</u>$_3$), 3 84 (s, 3H, OC<u>H</u>$_3$), 5 89 (m, 1H, <u>H</u>CO), 7 06-7 41 (m, 6H, arom <u>H</u> + =C<u>H</u>) ppm, ^{13}C-NMR ($CDCl_3$, 25 2 MHz) δ 10 5 (q, <u>C</u>H$_3$), 28 6 (q, <u>C</u>H$_3$), 53 1 (q, O<u>C</u>H$_3$), 71 5 (s, <u>C</u>C$_4$), 80 8 (d, H<u>C</u>O), 128 4, 128 5, 128 7 (3xd, Ph), 131 7 (s, Ph)*, 132 7 (s, =<u>C</u>CH$_3$)*, 146 8 (d, =<u>C</u>H), 168 6 (s, O<u>C</u>=O), 173 2 (s, O<u>C</u>=O), 201 3 (s, O=<u>C</u>CH$_3$) ppm (* signals may be interchanged), IR (CCl_4) ν 1772 (C=O, lactone), 1722 and 1715 (C=O, ester, acetyl) cm^{-1}, Mass data were the same as for diastereomer **15a**, HRMS/EI m/z calcd for $C_{16}H_{16}O_5$ 288 0998 Found 288 0997±0 0014

Methyl 3 (4-methyl-5-oxo-2,5-dihydro furan-2-yloxy)-2-phenyl-but-2-enoate (**2b**)

This compound was prepared according to procedure B, starting from **9** (269 mg, 1 38 mmol) The ratio of isomers **15** and **2b** was 3 7, according to ^{1}H-NMR analysis Purification by flash chromatography (SiO_2, eluens gradient hexane/ethyl acetate 9 1, 6 1, 3 1) afforded pure diastereomer **15a** as a white solid and **2b** as a colorless oil, which was slightly contaminated with diastereomer **15b** Total yield of **15b** and **2b** 69% R_f 0 18 (hexane/ethyl acetate 3 1), ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 2 01 (m, 6H, 2 C<u>H</u>$_3$), 3 68 (s, 3H, OC<u>H</u>$_3$), 6 21 (m, 1H, OC<u>H</u>O), 7 05 (m, 1H, =C<u>H</u>), 7 33 (m, 5H, arom <u>H</u>) ppm, ^{13}C-NMR ($CDCl_3$, 25 2 MHz) δ 10 5 (q, <u>C</u>H$_3$), 17 6 (q, <u>C</u>H$_3$), 51 8 (q, O<u>C</u>H$_3$), 99 9 (d, O<u>C</u>HO), 120 0 (s, =<u>C</u>CO$_2$Me), 127 7, 128 4, 129 6 (3xd, Ph), 134 1 (s, Ph)*, 134 9 (s, =<u>C</u>CH$_3$)*, 142 7 (d, =<u>C</u>H), 158 0 (s, =<u>C</u>O), 166 8 (s, O<u>C</u>=O), 171 4 (s, O<u>C</u>=O) ppm (* signals may be interchanged), IR (CCl_4) ν 1782 (C=O, lactone), 1720 (C=O, ester) cm ^{1},

3' Acetyl-4-methyl-5'-phenyl-4',5'-dihydro-2H,3'H-[2,3']bifuranyl-5,2'-dione (**16**)

To a stirred solution of γ-phenyl γ-butyrolactone (3 30 g, 20 4 mmol) and methyl acetate (3 2 g, 43 mmol) in DMF (50 mL) at 0^{0}C was added potassium *tert*-butoxide (2 54 g, 22 6 mmol) in a nitrogen atmosphere The mixture was allowed to warm to room temperature and stirring was continued 24 h Work-up of a small sample showed the presence of **10**, having identical spectroscopic properties as reported previously [23] The reaction mixture was cooled to -65^{0}C, followed by dropwise addition of a solution of **8a** (4 17 g, 23 6 mmol) in DMF (5 mL) and treated similarly as described in the general procedure (*vide supra*) Purification by flash chromatography (SiO_2, eluens gradient hexane/ethyl acetate 6 1, 4 1, 2 1) gave three fractions of different R_f-value containing compound **16** in a yield of 34%

Diastereomers **16a** and **16b** Obtained as a white solid after trituration with di*iso*propyl ether Yield 6%, diastereomeric ratio **16a** **16b** 1 5 according to ^{1}H-NMR analysis An analytically pure sample was obtained after recrystallization from 2-propanol, which did not affect the diastereomeric ratio Mp 115-124^{0}C, IR (KBr) ν 1762 (C=O, lactone), 1749 (C=O, lactone), 1708 (C=O, acetyl) cm ^{1}, MS (EI, m/z, rel int (%)) 300 ($[M]^+$, 3 2), 258 ($[C_{15}H_{14}O_4]^+$, 6 4), 203 ($[C_{12}H_{11}O_3]^+$, 93 6), 97 ($[C_5H_5O_2]^+$, 41 4), 43 ($[C_2H_3O]^+$, 100), Analysis calcd for $C_{17}H_{16}O_5$ C, 67 99, H, 5 36 Found C, 67 92, H, 5 31

Diastereomer **16a** R_f 0 25 (hexane/ethyl acetate 3 1), ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 1 98 (m, 3H, =CC<u>H</u>$_3$), 2 40 (s, 3H, C<u>H</u>$_3$), 2 68-2 89 (m, 2H, C<u>H</u>$_2$), 5 58 (m, 1H, <u>H</u>CO), 5 65 (t, 1H, J 8 2 Hz, PhC<u>H</u>O), 7 18 (m, 1H, =C<u>H</u>), 7 37 (m, 5H, arom <u>H</u>) ppm, ^{13}C-NMR ($CDCl_3$, 25 2 MHz) δ

10.8 (q, $\underline{C}H_3$), 26.4 (q, $\underline{C}H_3$), 36.6 (t, $\underline{C}H_2$), 64.7 (s, $\underline{C}C_4$), 80.2 (d, H$\underline{C}$O), 81.6 d, H$\underline{C}$O), 125.3, 129.0, (2xd, Ph), 132.2 (s, Ph)*, 138.3 (s, =$\underline{C}CH_3$)*, 145.3 (d, =$\underline{C}$H), 171.4 (s, O$\underline{C}$=O), 172.5 (s, O$\underline{C}$=O), 200.2 (s, O=$\underline{C}CH_3$) ppm (*: signals may be interchanged)

Diastereomer **16b**: R_f 0.20 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.82 (dd, 1H, ^{2}J 14.0 Hz, J 8.9 Hz, C$\underline{H}_2$), 1.94 (m, 3H, =CC$\underline{H}_3$), 2.57 (s, 3H, C$\underline{H}_3$), 3.07 (dd, 1H, ^{2}J 14.0 Hz, J 6.0 Hz, C$\underline{H}_2$), 5.53 (m, 1H, PhC$\underline{H}$O), 5.79 (m, 1H, $\underline{H}$CO), 7.01 (m, 1H, =C$\underline{H}$), 7.38 (m, 5H, arom. $\underline{H}$) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.8 (q, $\underline{C}H_3$), 26.1 (q, $\underline{C}H_3$), 33.5 (t, $\underline{C}H_2$), 65.6 (s, $\underline{C}C_4$), 80.5 (d, H$\underline{C}$O), 80.6 d, H$\underline{C}$O), 125.3, 126.0, (2xd, Ph), 133.7 (s, Ph)*, 138.1 (s, =$\underline{C}CH_3$)*, 143.6 (d, =$\underline{C}$H), 170.8 (s, O$\underline{C}$=O), 172.4 (s, O$\underline{C}$=O), 198.5 (s, O=$\underline{C}CH_3$) ppm (*: signals may be interchanged)

Diastereomer **16c**: Obtained as a white solid as one diastereomer. Yield 25%. An analytically pure sample was obtained after recrystallization from 2-propanol. Mp 88-89^{0}C; R_f 0.15 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.02 (m, 3H, C$\underline{H}_3$), 2.27 (dd, 1H, ^{2}J 14.4 Hz, J 7.9 Hz, C$\underline{H}_2$), 2.38 (s, 3H, C$\underline{H}_3$), 2.56 (dd, 1H, ^{2}J 14.4 Hz, J 8.0 Hz, C$\underline{H}_2$), 5.52 (t, 1H, J 7.9 Hz, PhC$\underline{H}$O), 5.60 (m, 1H, $\underline{H}$CO), 7.13 (m, 1H, =C$\underline{H}$), 7.30-7.43 (m, 5H, arom. $\underline{H}$); ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.8 (q, $\underline{C}H_3$), 26.8 (q, $\underline{C}H_3$), 32.7 (t, $\underline{C}H_2$), 64.3 (s, $\underline{C}C_4$), 79.7 (d, H$\underline{C}$O), 80.7 d, H$\underline{C}$O), 125.2, 126.9, 127.0 (3xd, Ph), 133.6 (s, Ph)*, 138.5 (s, =$\underline{C}CH_3$)*, 144.8 (d, =$\underline{C}$H), 172.3 (s, O$\underline{C}$=O), 172.6 (s, O$\underline{C}$=O), 200.0 (s, O=$\underline{C}CH_3$) ppm (*: signals may be interchanged); IR (KBr): ν 1761 (C=O, lactone), 1755 (C=O, lactone), 1708 (C=O, acetyl) cm^{-1}; MS (EI, m/z, rel. int. (%)): 300 ([M]$^+$, 3.6), 258 ([$C_{15}H_{14}O_4$]$^+$, 30.9), 203 ([$C_{12}H_{11}O_3$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 37.8), 43 ([C_2H_3O]$^+$, 66.5); Analysis calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.36. Found: C, 67.80; H, 5.25

Diastereomer **16d**: Obtained as a white solid after trituration with di*iso*propyl ether as one diastereomer. Yield 3%. An analytically pure sample was obtained after recrystallization from *iso*-propanol. Mp 149^{0}C; R_f 0.10 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.78 (dd, 1H, ^{2}J 13.6 Hz, J 9.9 Hz, C$\underline{H}_2$), 1.92 (m, 3H, C$\underline{H}_3$), 2.49 (s, 3H, C$\underline{H}_3$), 2.85 (dd, 1H, ^{2}J 13.6 Hz, J 6.8 Hz, C$\underline{H}_2$), 5.39 (dd, 1H, J 6.7 Hz, J 9.9 Hz, PhC$\underline{H}$O), 5.68 (m, 1H, $\underline{H}$CO), 6.95 (m, 1H, =C$\underline{H}$), 7.33-7.43 (m, 5H, arom. $\underline{H}$); ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.8 (q, $\underline{C}H_3$), 27.1 (q, $\underline{C}H_3$), 32.7 (t, $\underline{C}H_2$), 66.0 (s, $\underline{C}C_4$), 79.0 (d, H$\underline{C}$O), 79.7 d, H$\underline{C}$O), 125.8, 129.0, 129.2 (3xd, Ph), 133.8 (s, Ph)*, 137.9 (s, =$\underline{C}CH_3$)*, 143.7 (d, =$\underline{C}$H), 171.4 (s, O$\underline{C}$=O), 172.1 (s, O$\underline{C}$=O), 198.7 (s, O=$\underline{C}CH_3$) ppm (*: signals may be interchanged); IR (KBr): ν 1763 (C=O, lactone), 1719 (C=O, acetyl) cm^{-1}; MS (EI, m/z, rel. int. (%)): 300 ([M]$^+$, 2.7), 257 ([$C_{15}H_{13}O_4$]$^+$, 7.9), 203 ([$C_{12}H_{11}O_3$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 37.8), 43 ([C_2H_3O]$^+$, 83.2); Analysis calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.36. Found: C, 67.49; H, 5.31

3-[1-(4-Methyl-5-oxo-2,5-dihydro-furan-2-yloxy)-ethylidene]-3,3a,4,8b-tetrahydro-indeno[1,2-b]furan-2-one (**4b**) *and 3-acetyl-3-(4-methyl-5-oxo-2,5-dihydro-furan-2-yl)-3,3a,4,8b-tetrahydro-indeno[1,2-b]furan-2-one* (**17**)

These compounds were prepared following procedure B, starting from **11** (568 mg, 2.63 mmol). The ratio of isomers **4b** and **17** in the crude reaction mixture was 1:2, according to ^{1}H-NMR analysis. Purification by flash chromatography (SiO_2, eluens gradient hexane/ethyl acetate 6:1, 3:1 and 1:1) afforded three fractions containing O- and C-alkylated products

Compound **17**: Obtained as a white solid (299 mg, 36%) as a mixture of two diastereomers (ratio 3:1). An analytically pure sample of the main diastereomer was obtained after recrystallization from hexane/ethyl acetate. Mp 173-176^{0}C; R_f 0.18 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.84 (s, 3H, C$\underline{H}_3$ D-ring), 2.02 (m, 3H, C$\underline{H}_3$), 2.77 (dd, 1H, ^{2}J 17.1 Hz, J 2.9 Hz,

$C\underline{H}_2$), 2.97 (m, 1H, $CH_2C\underline{H}$), 3.14 (dd, 1H, ^2J 17.1 Hz, J 8.8 Hz, $C\underline{H}_2$), 5.35 (m, 1H, $\underline{H}CO$), 5.94 (d, 1H, J 7.5 Hz, $PhC\underline{H}O$), 7.20 (m, 1H, arom. $\underline{H}$), 7.35 (m, 2H, arom. $\underline{H}$), 7.46 (m, 1H, $=C\underline{H}$), 7.53 (m, 1H, arom. $\underline{H}$); ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.9 (q, $\underline{C}H_3$), 29.9 (q, $\underline{C}H_3$), 33.4 (t, $\underline{C}H_2$), 41.3 (d, $CH_2\underline{C}H$), 66.0 (s, $\underline{C}C_4$), 80.4 (d, $H\underline{C}O$), 85.9 d, $H\underline{C}O$), 125.0, 126.1, 128.2, 130.4 (4xd, Ph), 133.6 (s, Ph)*, 138.3 (s, Ph)*, 141.8 (s, $=\underline{C}CH_3$)*, 145.5 (d, $=\underline{C}H$), 172.4 (s, $O\underline{C}=O$), 173.9 (s, $O\underline{C}=O$), 202.5 (s, $O=\underline{C}CH_3$) ppm (*: signals may be interchanged); IR (KBr): ν 1760 (C=O, lactone), 1695 (C=O, acetyl) cm^{-1}; GC-MS (EI, m/z, rel. int. (%)): 313 ([M+1]$^+$, 3.1), 270 ([$C_{16}H_{14}O_4$]$^+$, 8.3), 215 ([$C_{13}H_{11}O_3$]$^+$, 20.2), 97 ([$C_5H_5O_2$]$^+$, 5.5), 43 ([C_2H_3O]$^+$, 100); Analysis calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.29; H, 5.15

Fast moving diastereomer **4b**: Obtained as a yellow solid (88 mg, 11%). An analytical sample was obtained after recrystallization from hexane/ethyl acetate as white crystals. Mp 144-148^{0}C; R_f 0.12 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.96 (m, 3H, $C\underline{H}_3$ D-ring), 2.24 (d, 3H, ^5J 1.2 Hz, $C\underline{H}_3$), 3.02 (dd, 1H, ^2J 16.4 Hz, J 4.0 Hz, $C\underline{H}_2$), 3.58 (dd, 1H, ^2J 16.4 Hz, J 9.3 Hz, $C\underline{H}_2$), 3.92 (m, 1H, $CH_2C\underline{H}$), 5.86 (d, 1H, J 7.7 Hz, $PhC\underline{H}O$), 6.15 (m, 1H, $OC\underline{H}O$), 7.15 (m, 1H, $=C\underline{H}$), 7.29-7.54 (m, 4H, arom. $\underline{H}$); ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.6 (q, $\underline{C}H_3$), 20.2 (q, $\underline{C}H_3$), 39.6 (t, $\underline{C}H_2$), 40.7 (d, $CH_2\underline{C}H$), 84.3 (d, $H\underline{C}O$), 101.5 (d, $O\underline{C}HO$), 114.9 (s, $\underline{C}=C(O)CH_3$), 125.1, 126.5, 127.6, 130.2 (4xd, Ph), 134.1 (s, Ph)*, 138.9 (s, Ph)*, 142.3 (s, $=\underline{C}CH_3$)*, 143.1 (d, $=\underline{C}H$), 163.0 (s, $\underline{C}=O$)*, 167.6 (s, $\underline{C}=O$)*, 171.5 (s, $=\underline{C}(O)CH_3$)* ppm (*: signals may be interchanged); IR (CCl_4): ν 1780 (C=O, lactone), 1750 and 1725 (C=O, lactone) cm^{-1}; Analysis calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.27; H, 5.14.

Slow moving diastereomer **4b**: Obtained as a yellow solid (61 mg, 7%). An analytical sample was obtained after recrystallization from hexane/ethyl acetate as slightly yellow crystals. Mp 164-167^{0}C; R_f 0.05 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.97 (m, 3H, $C\underline{H}_3$ D-ring), 2.25 (d, 3H, ^5J 1.2 Hz, $C\underline{H}_3$), 3.05 (dd, 1H, ^2J 16.6 Hz, J 4.7 Hz, $C\underline{H}_2$), 3.57 (dd, 1H, ^2J 16.6 Hz, J 9.5 Hz, $C\underline{H}_2$), 3.93 (m, 1H, $CH_2C\underline{H}$), 5.85 (d, 1H, J 7.8 Hz, $PhC\underline{H}O$), 6.21 (m, 1H, $OC\underline{H}O$), 7.15 (m, 1H, $=C\underline{H}$), 7.25-7.38 (m, 3H, arom. $\underline{H}$), 7.51 (d, 1H, J 7.5 Hz, arom. $\underline{H}$) ppm; IR (CCl_4): ν 1780 (C=O, lactone), 1750 and 1725 (C=O, lactone) cm^{-1}; MS (EI, m/z, rel. int. (%)): 312 ([M]$^+$, 0.23), 215 ([$C_{13}H_{11}O_3$]$^+$, 38.7), 97 ([$C_5H_5O_2$]$^+$, 100).; Analysis calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.39; H, 5.06

Methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydro-furan-2-yloxy)-but-2-enoate (**5b**) *and methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-2-(4-methyl-5-oxo-2,5-dihydro-furan-2-yl)-3-oxo-butyrate* (**18**)

The reaction was carried out following procedure A, starting from **12** (0.63 g, 2.4 mmol). The ratio of isomers **5b** and **18** in the crude reaction mixture was 2:3, according to ^{1}H-NMR analysis. Purification by flash chromatography (SiO_2, eluens hexane/ethyl acetate 1:1) gave **5b** and **18** (ratio of diastereomers **18a**:**18b** 6:1) as pale yellow solids in a yield of 67%.

Diastereomers **18a** and **18b**: Recrystallized from 2-propanol to give analytically pure **18a** and **18b** (ratio 6:1) as white crystals. Mp 181-183^{0}C; IR (KBr): ν 1760 (C=O, lactone), 1720 (C=O, imide, acetyl, ester) cm^{-1}; MS (EI, m/z, rel. int. (%)): 357 ([M]$^+$, 0.1), 315 ([$C_{16}H_{13}NO_6$]$^+$, 58.1), 260 ([$C_{13}H_{10}NO_5$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 7.0), 43 ([C_2H_3O]$^+$, 44.9); Analysis calcd for $C_{18}H_{15}NO_7$: C, 60.51; N, 3.92; H, 4.23. Found: C, 60.44; N, 3.83; H, 4.14

Diastereomer **18a**: R_f 0.32 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.75 (m, 3H, $=CC\underline{H}_3$), 2.35 (s, 3H, $C\underline{H}_3$), 3.92 (s, 3H, $OC\underline{H}_3$), 6.20 (m, 1H, $\underline{H}CO$), 7.15 (m, 1H, $=C\underline{H}$), 7.64 (m, 4H, arom. $\underline{H}$) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.5 (q, $\underline{C}H_3$), 26.8 (q, $\underline{C}H_3$), 54.1 (q, $O\underline{C}H_3$), 73.3 (s, $N\underline{C}C_3$), 80.6 (d, $H\underline{C}O$), 124.0 (d, Ph), 130.8 (s, Ph)*, 131.4 (s, $=\underline{C}CH_3$)*,

135.0 (d, Ph), 145.9 (d, =CH), 163.8 (s, OC=O), 166.9 (s, NC=O), 172.7 (s, OC=O), 197.5 (s, O=CCH$_3$) ppm (*: signals may be interchanged)
Diastereomer **18b**: R_f 0.32 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.75 (m, 3H, =CCH$_3$), 2.39 (s, 3H, CH$_3$), 3.87 (s, 3H, OCH$_3$), 6.20 (m, 1H, HCO), 7.15 (m, 1H, =CH), 7.64 (m, 4H, arom. H) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.5 (q, CH$_3$), 26.7 (q, CH$_3$), 53.8 (q, OCH$_3$), 73.3 (s, NCC$_3$), 79.6 (d, HCO), 124.0 (d, Ph), 130.8 (s, Ph)*, 131.4 (s, =CCH$_3$)*, 135.0 (d, Ph), 146.2 (d, =CH), 163.8 (s, OC=O), 166.9 (s, NC=O), 172.7 (s, OC=O), 195.2 (s, O=CCH$_3$) ppm (*: signals may be interchanged)
Compound **5b**: Recrystallized from 2-propanol/dichloromethane to give **5b** as white crystals. Mp 195-196.5^{0}C; R_f 0.20 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.89 (m, 3H, =CCH$_3$ D-ring), 2.82 (s, 3H, CH$_3$), 3.70 (s, 3H, OCH$_3$), 6.31 (m, 1H, OCHO), 6.71 (m, 1H, =CH), 7.73-7.96 (m, 4H, arom. H) ppm; ^{13}C-NMR ($CDCl_3$, 25.2 MHz): δ 10.6 (q, CH$_3$), 15.6 (q, CH$_3$), 52.2 (q, OCH$_3$), 96.5 (d, OCHO), 105.5 (s, =CCO$_2$Me), 123.5, 123.8 (2xd, Ph), 132.1 (s, Ph)*, 134.2 (d, Ph), 134.7 (s, =CCH$_3$)*, 141.7 (d, =CH), 164.0 (s, =CO), 165.8 (s, NC=O), 166.6 (s, OC=O), 170.5 (s, OC=O) ppm (*: signals may be interchanged); IR (KBr): ν 1774 (C=O, lactone), 1718 (C=O, ester), 1698 (C=O, imide), 1638 (C=C, enol ether) cm^{-1}; MS (EI, m/z, rel. int. (%)): 357 ([M]$^+$, 0.4), 260 ([$C_{13}H_{10}NO_5$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 21.7); Analysis calcd for $C_{18}H_{15}NO_7$: C, 60.51; N, 3.92; H, 4.23. Found: C, 60.78; N, 3.92; H, 4.15

Irradiation of Z-methyl 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydro-furan-2-yloxy)-acrylate (**5a**)
This reaction was carried out similarly as described by MacAlpine *et al.*.[31] A solution of Z-isomer **5a** (500 mg, 1.46 mmol) in dry toluene (200 mL) was irradiated for a few hours in the presence of benzophenone (200 mg). The solution was concentrated *in vacuo* and subsequently passed over a short column of silica to remove benzophenone. A 1:1 mixture of **6** and **5a** in quantitative yield was thus obtained, having identical R_f-values on TLC (eluens: hexane/ethyl acetate 1:1). The crude mixture was triturated with ethyl acetate, from which only the *E*-isomer **6** precipitated. Analytically pure **6** (175 mg, 35 %) was obtained by recrystallization from 2-propanol as white crystals. Mp 197-200^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.04 (m, 3H, CH$_3$), 3.74 (s, 3H, OCH$_3$), 6.22 (br s, 1H, OCHO), 7.07 (br s, 1H, =CH), 7.10 (s, 1H, =CHO), 7.72-7.97 (m, 4H, arom. H) ppm; Analysis calcd for $C_{17}H_{13}NO_7$: C, 59.48; H, 3.82; N, 4.08. Found: C, 59.34; H, 3.76; N, 4.10.

Irradiation of (Z)-methyl 3-(4-methyl-5-oxo-2,5-dihydro-furan-2-yloxy)-2-phenyl-but-2-enoate (**2b**)
A solution of Z-isomer **2b** (194 mg, 0.67 mmol), slightly contaminated with **15**, in acetone (10 mL) was irradiated for 30 min. in the presence of benzophenone (31 mg, 0.17 mmol). The solution was concentrated *in vacuo* and subsequently passed over a short column of silica (eluens dichloromethane) to remove benzophenone. An inseparable mixture of **2b** and **2c** (ratio 5:3) in almost quantitative yield was thus obtained as a colorless oil, having identical R_f-values on TLC using several eluentia.
Relevant ^{1}H-NMR data ($CDCl_3$, 400 MHz) of **4c**: δ 1.86 (m, 3H, CH$_3$ D-ring), 2.56 (s, 3H, CH$_3$), 3.68 (s, 3H, OCH$_3$), 6.05 (br s, 1H, OCHO), 6.45 (br s, 1H, =CH) ppm.

Biological activity

Seeds

Seeds of *Striga hermonthica* (from *Sorghum bicolor* (L.) Moench) and *Orobanche crenata* (from *Vicia faba* L.) were harvested in Burkina Faso in 1994 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests. Bioassays were carried out essentially following the procedure of Mangnus *et al.* [33] with minor modifications.

Preparation of test solutions

A compound to be tested was weighed out very accurately to the amount of 2.5 mg, dissolved in 5 mL of acetone p.a. and diluted with demineralized water to 25 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 2, 1, 0.1 and 0.01 mg/L test compound and 0.4, 0.2, 0.02 and 0.002% (v/v) acetone, respectively.

Bioassays

For surface sterilization seeds of *Striga hermonthica* and *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight. For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30-70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20°C for *Orobanche* seeds and at 30°C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at the indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.2, 0.02 and 0.002% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (concentrations of 1 and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 (*Striga*) or 9 (*Orobanche*) disks per treatment.

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Chapter 12*

Synthesis and Biological Evaluation of Potential Substrates for the Isolation of the Strigol Receptor

Abstract: A new series of analogs of strigol, which is a germination stimulant for seeds of the parasitic weeds *Striga* and *Orobanche*, has been prepared. For the isolation and characterization of the strigol-receptor, labeled analogs are required in which a photoreactive function may be incorporated. The synthesis of a range of A-ring substituted analogs of GR24, including fluorescent dansyl GR24 **12** has been accomplished. Bioassays revealed that the stimulatory activity of these analogs in the seed germination of *Striga hermonthica* is retained.

12.1 Introduction

Parasitic weeds belonging to the genera *Striga* and *Orobanche* severely reduce yields of economically important crops in tropical and semitropical areas of the eastern hemisphere.[1,2] The parasitic process begins with the seed germination of the weeds, induced by a stimulant which is present in the root exudate of the host plant. Following the isolation and identification of the naturally occurring germination stimulant (+)-strigol (fig 1),[3,4] several structure-bioactivity studies have been conducted, which revealed that the bioactiphore resides in the CD-fragment and the vinyl ether moiety.[5-10]

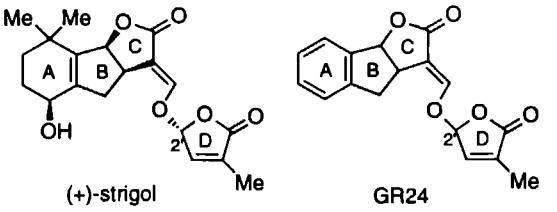

Figure 1

In particular GR24 (fig. 1) turned out to be a highly potent synthetic strigol analog.[5,6,11] Based on the structural requirements for retaining stimulatory activity a tentative molecular mechanism for germination has been proposed[8] which is depicted in scheme 1.

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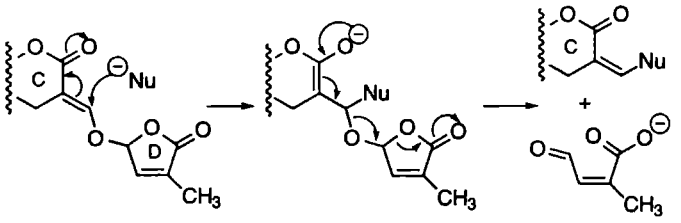
Scheme 1



According to this mechanism a nucleophilic site in the receptor cavity reacts with the Michael acceptor unit, followed by elimination of the D-ring. The C- and D-rings as well as the connecting vinyl ether unit play an important role in inducing the germination. It is thus suggested that the chemical reaction at the receptor site is of crucial importance at the very beginning of the signal-transduction chain. However, nothing is known about the structure of the receptor protein nor of its localization within the seeds. Detailed knowledge of the receptor protein would enable the design of a perfectly fitting substrate. Current strategies in the purification of plant proteins involve several types of affinity chromatography and photoaffinity labeling.[12] In photoaffinity labeling the substrate of the protein is converted to a photoaffinity ligand by covalently attaching a photoreactive moiety, such as an azido group, to the natural ligand.[13] After exposure to a cell extract, a very short-living, highly reactive intermediate is generated upon irradiation, which will be covalently bonded onto the protein in the vicinity of the putative ligand-binding site. A radioactive or fluorescent tag can be introduced in the photoreactive ligand in order to allow detection during isolation and enable the characterization of the protein.[14] It was demonstrated that this technique is a useful tool in plant hormone research, exemplified by the photoaffinity labeling of auxin binding proteins[15] and gibberellin binding proteins.[16] In the case of the "strigol receptor", incorporation of a photoreactive moiety may not strictly be necessary. If the molecular mechanism (scheme 1) is correct, treating the seeds with a radioactive or fluorescent strigol analog may directly lead to covalent attachment to the receptor protein without the need to generate a highly reactive species by irradiation. Knowing the bioactiphore of the ligand, incorporation of the tag and eventually a photolabile moiety should be such that the bioactivity is retained. Structure-activity relationship studies, which have been performed previously[8-10] will therefore provide a firm basis for the design of suitable compounds for the isolation of the 'strigol receptor'.

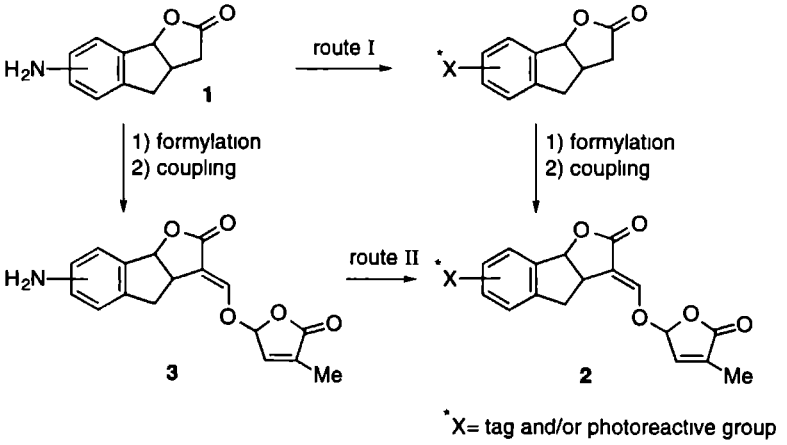
In this chapter synthetic approaches are described for the preparation of biologically active, labeled strigol analogs, suitable for, at least in principle, the identification of the strigol receptor. The synthetic concept is aimed to provide a general approach to a wide range of substrates containing a tag and eventually an additional photolabile moiety. In addition, the activity of the stimulation of seed germination of *Striga hermonthica* (Del.) Benth. and *Orobanche crenata* Forsk. of some of the thus obtained strigol analogs is evaluated.

12.2 Results and discussion

Strategy

In designing potential, labeled germination stimulants, GR24 (fig 1) was used as the lead molecule The bioactivity of GR24 is very high and its preparation is well documented [11] The CD-part as well as the connecting enol ether unit are essential for full biological activity,[8,17] therefore it was figured that the tag and/or photoreactive group can be best incorporated in the A-ring of the stimulant molecule Amino tricyclic lactone **1** was selected as an appropriate compound to serve this purpose as a wide range of transformations are feasible with this synthon, such as acylation, sulfonylation or alkylation reactions whereby coupling with an external tag can be achieved The strategy for the synthesis of labeled GR24 analogs is outlined in scheme 2 The label may be introduced either before (route I) or after (route II) coupling with the D-ring

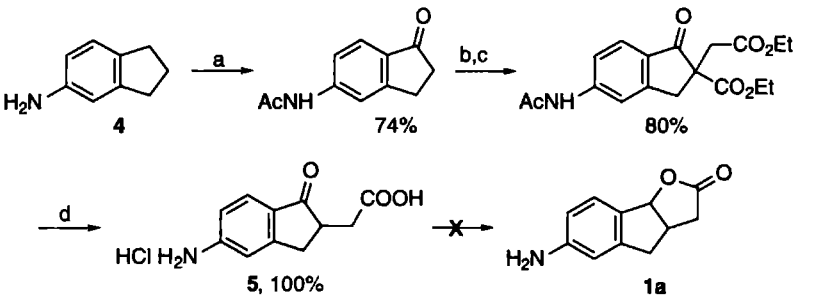
Scheme 2



Synthesis

The preparation of key intermediate 6-amino tricyclic lactone **1a** was attempted starting from commercially available 6-amino indane **4** (scheme 3) Conversion into the known *N*-acetyl-6-amino-1-indanone,[18] followed by carboxylation, alkylation and decarboxylation essentially as described for the synthesis of GR24[11] afforded amino acid **5** in high overall yield However, all attempts to reduce the keto function to achieve lactonization failed Therefore, the synthetic strategy was reconsidered and tricyclic lactone **6**[11] was chosen as the starting material, whereby the introduction of the amino function in a regiocontrolled manner is the main issue (scheme 4)

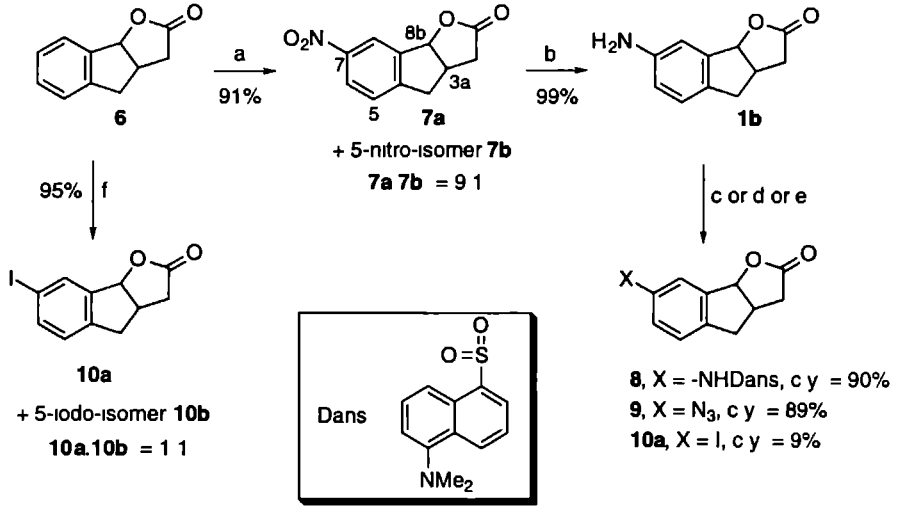
Scheme 3



a) 1 Ac_2O, DMAP 2 CrO_3, H_2SO_4 b) NaH (2 2 equiv), $(EtO)_2CO$
c) NaH (1 1 equiv), $BrCH_2CO_2Et$ d) HOAc, HCl (aq), Δ

Nitration of **6** under relatively mild conditions[19] afforded two regioisomeric lactones **7a** and **7b** in a 9 1 ratio and in excellent yield These lactones could readily be separated by chromatography The structure of **7a** was deduced unambigiously from a 2D-NOESY experiment The preferred formation of **7a** can be explained by the fact that C_7 is the least electron-deficient carbon atom in the aromatic ring Reduction of the nitro group was accomplished employing aqueous $Cu(OAc)_2$ and $NaBH_4$ in methanol[20] in yields ranging from 34 to 95% A more reliable procedure involves the use of Sn-HCl under reflux conditions,[21] which gave 7-amino tricyclic lactone **1b** in a reproducible yield of 99% (77% after recrystallization)

Scheme 4

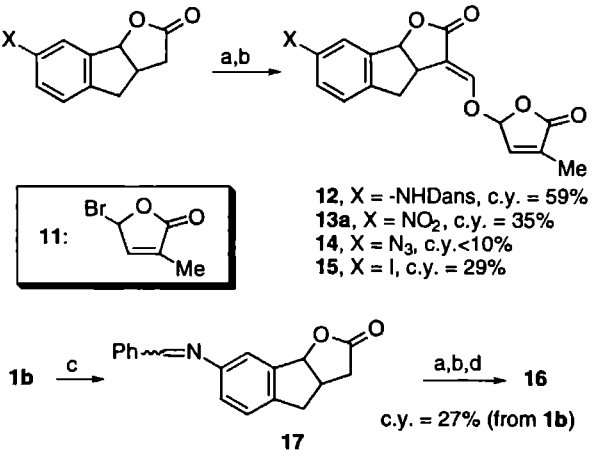


a) $NaNO_3$, TFA, b) Sn-HCl (aq), Δ, c) X = -NHDans pyridine, DansCl,
d) X = N_3 1 $NaNO_2$, TFA 2 NaN_3, e) X = I 1 $NaNO_2$, TFA 2 KI, f) I_2, $PhI(OC(O)CF_3)_2$

The introduction of a fluorescent group (dansyl), photolabile group (N_3) and iodine was then investigated (scheme 4). The synthesis of fluorescent 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) tricyclic lacone **8** and azido tricyclic lactone **9** proceeded smoothly via established procedures. Dansyl tricyclic lactone **8** was formed upon treatment of amine **1b** with dansyl chloride in pyridine.[22] Azido tricyclic lactone **9** was prepared via diazotation and subsequent treatment with NaN_3.[23] In contrast, iodination of **1b** via diazotation gave the iodo tricyclic lactone **10a** in only 9% yield. However, this iodo lactone **10a** could be obtained in a direct manner from tricyclic lactone **6** employing iodine in the presence of bis(trifluoroacetoxy)phenyliodide (scheme 4) by adopting the procedure of Merkushev *et al.* [24] An 1:1 mixture of regioisomeric 7-iodo lactone **10a** and 5-iodo lactone **10b** was obtained in an excellent yield. These iodides could readily be separated by chromatography. Structural assignments were performed by comparison of the ^{1}H-NMR spectra with those of the 7-nitro and 5-nitro counterparts **7a** and **7b**, respectively.

Coupling reactions to give the desired GR24 analogs via route I (scheme 2) involve a two step procedure, similarly as described for the synthesis of GR24.[11] First formylation and then quenching of the intermediate hydroxymethyleno anion with bromo butenolide **11** in an one pot procedure provides a mixture of two diastereomeric GR24 adducts. The results of the coupling reactions of some 7-substituted tricyclic lactones are summarized in scheme 5.

Scheme 5



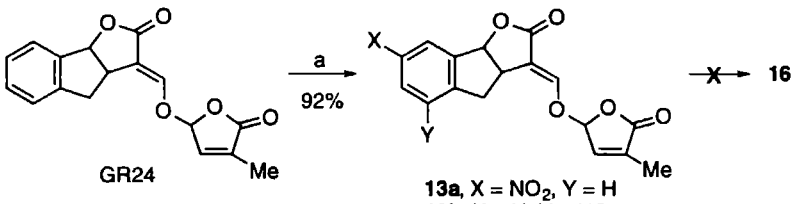
a) KOtBu, HCOOMe b) bromobutenolide **11**
c) PhCHO, mol. sieves d) SiO_2

The overall yields of these reactions are low to moderate. A possible explanation for these results is that the formylation reactions were not complete according to TLC analysis. Consequently, purification of dansyl GR24 **12** and nitro GR24 **13a** by recrystallization was

hampered by the presence of small amounts of starting material. Therefore, it is more practical to isolate the intermediate hydroxymethyleno lactone, as was done in the case of the preparation of iodo GR24 **15**. Purification of the intermediate then can simply be accomplished by washing with diethyl ether to remove unreacted starting material, although some loss of material has to be accepted The product after coupling, *viz* iodo GR24 **15**, could now readily be purified by chromatography and recrystallization It was not possible to prepare azido GR24 **14** and amino GR24 **16** by this procedure The amino function in **1b** caused problems in the formylation reaction Therefore, the amino group was first protected as a Schiff base by reaction with benzaldehyde, then followed by formylation, coupling with bromo butenolide **11** and finally deprotection (scheme 5) Crude 7-imino GR24 **17** was isolated in an overall yield of 78%, based on **1b** Deprotection was not as straightforward as expected Several conventional methods, *e g* using 5% oxalic acid, failed to give the desired product and only starting imine was recovered However, during purification of 7-benzalimino GR24 by flash chromatography, deprotection took place on the silica gel column and amino GR24 **16** was isolated in an overall yield of 27% The diastereomers could not be separated, neither by flash chromatography nor by recrystallization in contrast to GR24 analogs **12**, **13** and **15**

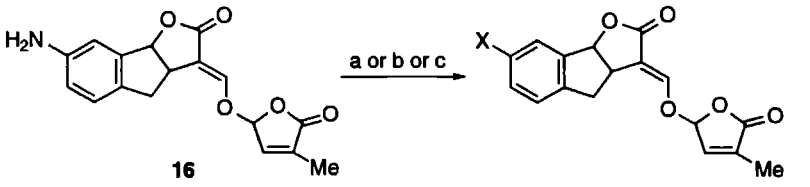
Due to the problems encountered in the synthesis and purification of some GR24 analogs, especially **12** and **13**, the sequence of events was revised and essentially route II (scheme 2) was followed In this manner, nitro GR24 **13** was synthesized starting from GR24 in a highly efficient manner (scheme 6) Only negligible amounts of regioisomeric by-products were found However, several attempts to obtain amino GR24 **16** by reduction of the nitro function in **13** were not successful, and therefore for this compound route I (scheme 2) is more suitable

Scheme 6



Amino GR24 **16** was then utilized for further derivatization, especially for the purpose of receptor identification (scheme 7) Diazotation and substitution employing NaN_3 gave **14**, which is thermally rather unstable, in high yield Applying the appropriate sulfonyl chloride, mesyl GR24 **18** and dansyl GR24 **12** were synthesized in high yields Purification of the compounds prepared via this route II was much simpler than by route I (schemes 2 and 5), and is therefore preferred

Scheme 7



18, X = -NHMs, c.y. = 78%
12, X = -NHDans, c.y. = 89%
14, X = N_3, c.y. =88%

a) X = -NHMs: pyridine, MsCl
b) X = -NHDans: pyridine, DansCl
c) X = N_3: 1. $NaNO_2$, 80% HOAc 2. NaN_3

Biological activity

The stimulatory activity of nitro GR24 **13a**, amino GR24 **16** and dansyl GR24 **12** was determined using seeds of *Striga hermonthica* and *Orobanche crenata*. The germination percentages are collected in Tables I and II, together with those obtained for GR24 under the same conditions in the same bioassay. This reference to GR24 enables the comparison between results obtained in different test series, which is important, because the response of seeds of parasitic weeds, in particular of *Striga hermonthica*, varies considerably from test to test. For the bioassays of nitro GR24 **13a** and dansyl GR24 **12**, the fast moving diastereomers were used, whereas amino GR24 **16** was tested as a mixture of diastereomers. It was shown for GR24 that the activity of the most active (fast moving) diastereomer is not seriously influenced by the presence of the less active diastereomer.[11]

Table I. Germination percentages for seeds of *Striga hermonthica* after exposure to solutions of **12**, **13a** and **16** at different concentrations[a]

		% germination ± S.E. at		
entry	compound	1 mg/L	0.01 mg/L	0.001 mg/L
1	**12**[c]	70.0 ± 1.7 (48.4 ± 2.5)[b]	57.4 ± 2.5 (58.5 ± 2.4)[b]	21.2 ± 1.5 (24.3 ± 1.6)[b]
2	**13a**[c]	50.1 ± 4.0 (48.4 ± 2.5)[b]	62.6 ± 2.9 (58.5 ± 2.4)[b]	33.6 ± 3.6 (24.3 ± 1.6)[b]
3	**16**[d]	46.9 ± 6.6 (50.5 ± 6.1)[b]	20.7 ± 4.4 (67.5 ± 3.1)[b]	9.4 ± 2.4 (35.9 ± 5.1)[b]

a) Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions. Germination percentages given are means ± S.E. by counting *ca.* 550 seeds, distributed over 12 discs, of one representative experiment.
b) The values in parantheses are the mean germination percentages for seeds tested under the same conditions and at the same time, with GR24 as stimulant
c) Aqueous control (0.1 % v/v) acetone: 8.6 ± 1.1% germination
d) Aqueous control (0.1 % v/v) acetone: 10.8 ± 1.1% germination

Table II. Germination percentages for seeds of *Orobanche crenata* after exposure to solutions of **12**, **13a** and **16** at different concentrations[a]

		% germination ± S E at		
entry	compound	1 mg/L	0 1 mg/L	0 01 mg/L
1	**12**	3 0 ± 1 3	0 0 ± 0 0	0 0 ± 0 0
2	**13a**	54 8 ± 2 9	9 0 ± 1 5	0 6 ± 0 4
3	**16**	31 7 ± 3 5	1 4 ± 0 8	0 0 ± 0 0
4	control[b]	0 0 ± 0 0	0 0 ± 0 0	0 0 ± 0 0
5	GR24	57 0 ± 2 3	27 3 ± 2 0	0 3 ± 0 3

a) Activities are indicated as germination percentages after treatment of the seeds with stimulant solutions Germination percentages given are means ± S E by counting *ca* 550 seeds, distributed over 12 discs, of one representative experiment

b) Aqueous control containing 0 1, 0 01 and 0 001 % (v/v) acetone

The data in Tables I and II reveal that compounds **12**, **13a** and **16** behave quite differently toward seeds of *Striga hermonthica* and *Orobanche crenata* In the case of *Striga hermonthica*, the biological activity of the substituted GR24 derivatives is relatively little affected as compared to GR24 The activity of amino GR24 **16** is about one order of magnitude lower than GR24, whereas nitro GR24 **13a** and dansyl GR24 **12** possess comparable concentration dependent activities Interestingly, the intrinsic activity of dansyl GR24 **12** is considerably higher than that of GR24, which becomes apparent at the higher concentrations In contrast, dansyl GR24 **12** is completely inactive in the stimulation of *Orobanche crenata* seeds Evidently, substituents in the A-part of GR24 have a negative effect on the bioactivity in the case of this parasitic species The difference in response exerted by A-ring analogs **12**, **13a** and **16** on seeds of *Striga hermonthica* and *Orobanche crenata* is rather unexpected, since previous studies revealed that structural modifications in the BC-part,[9] enol ether moiety[25] and D-ring[26] generally give similar results for both parasitic species Nevertheless, the prospects of incorporating a tag in the A-ring of GR24 with the aim to identify the receptor protein is very promising for *Striga hermonthica* The remarkable activity of the bulky dansyl derivative **12** suggests a large degree of structural freedom in the A-part to retain full biological activity

12.3 Concluding remarks

In this synthetic study the preparation of amino GR24 **16** is described This compound is a versatile synthon for the preparation of a range of A-ring analogs of GR24 The synthesis of the fluorescent GR24 analog **12** has been accomplished Application of the thus developed synthetic strategy provides a feasible approach to the incorporation of radioactive tags and photoreactive units in the GR24 molecule The germination stimulatory activity of GR24 analogs **12**, **13a** and **16** is relatively little affected for seeds of *Striga hermonthica*, whereas it is considerably reduced

for seeds of *Orobanche crenata* Therefore *Striga hermonthica* is an attractive target to perform protein fishing experiments

12.4 Experimental section

Synthesis

General remarks

100 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard) and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me_4Si as internal standard) All coupling constants are given as ^3J in Hz, unless indicated otherwise IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer For mass spectra a double focussing VG7070E mass spectrometer was used GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system Separation was carried out on a fused-silica capillary column (DB-5, 30m x 0 25 mm) Helium was used as carrier gas, and electron impact (EI) was used as ionization mode GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0 5 atm) as the carrier gas Melting points were measured with a Reichert Thermopan microscope and are uncorrected Elemental analyses were performed at the Department of Micro-analysis of this laboratory Fluorescence measurements were performed using a Perkin-Elmer luminescence spectrometer LS50B

Solvents were dried using the following methods Dichloromethane was distilled from P_2O_5 Diethyl ether was distilled from NaH Hexane was distilled from CaH_2 Tetrahydrofuran was distilled from lithium aluminium hydride just before use All other solvents were of analytical grade Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0 25 mm) using the eluents indicated Spots were visualized with UV or using a molybdate spray Flash-chromatography was carried out at a pressure of *ca* 1 5 bar, using Merck Kieselgel 60H Column chromatography at atmospheric pressure was carried out, using Merck Kieselgel 60

3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one (**6**) and 5-bromo-3-methyl-2(5H)-furanone (**11**) were prepared according to known procedures [11]

7-Nitro-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**7 a**) and *5 nitro-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one* (**7b**)

Sodium nitrate (0 51 g, 6 0 mmol) was added to a solution of tricyclic lactone **6** (348 mg, 2 00 mmol) in TFA (15 mL) at room temperature The solution was stirred overnight TFA was removed *in vacuo*, and the residue was dissolved in ethyl acetate and saturated $NaHCO_3$ solution The aqueous phase was extracted with ethyl acetate (2x) The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a mixture of **7a** and **7b** (399 mg, 91%) as a solid The ratio of **7a** **7b** amounted to 9 1 as was determined by ^{1}H-NMR (100 MHz) analysis Pure **7a** (319 mg, 75%) was obtained by recrystallization from toluene The residue, containing **7b**, was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1 1) to give pure **7b** (40 mg, 9%) as a white solid An analytically pure sample was obtained by crystallization from hexane/ethyl acetate

7a Mp 118-119^{0}C, R_f 0 21 (hexane/ethyl acetate 1 1), ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2 43 (dd, 1H, ^2J 18 2 Hz, J 5 3 Hz, H_3), 2 96 (dd, 1H, ^2J 18 2 Hz, J 9 7 Hz, H_3), 3 00 (dd, 1H, ^2J 17 2 Hz,

J 3.4 Hz, H_4), 3.44 (dd, 1H, ^{2}J 17.2 Hz, J 8.6 Hz, H_4), 3.52 (m, 1H, H_{3a}), 5.94 (d, 1H, J 7.1 Hz, H_{8b}), 7.45 (d, 1H, J 8.4 Hz, H_5), 8.25 (dd, 1H, ^{4}J 2.1 Hz, J 8.4 Hz, H_6), 8.35 (d, 1H, ^{4}J 2.1Hz, H_8) ppm; MS (EI, m/z, rel. int. (%)): 219 ([M]$^+$, 39), 175 ([$C_{10}H_9NO_2$]$^+$, 100), 128 ([$C_{10}H_8$]$^+$, 84), 115 ([C_9H_7]$^+$, 33); Analysis calcd for $C_{11}H_9NO_4$: C, 60.28; H, 4.14; N, 6.39. Found: C, 60.12; H, 4.04; N, 6.37.

7b Mp 137-140^{0}C; R_f 0.35 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.46 (dd, 1H, ^{2}J 18.1 Hz, J 5.0 Hz, H_3), 2.90 (dd, 1H, ^{2}J 18.1 Hz, J 9.5 Hz, H_3), 3.29-4.00 (m, 3H, H_4 and H_{3a}), 5.95 (d, 1H, J 7.4 Hz, H_{8b}), 7.53 (t, 1H, J 7.7 Hz, H_7), 7.83 (d, 1H, J 7.7 Hz, H_8), 8.25 (d, 1H, J 7.7 Hz, H_6) ppm; MS (EI, m/z, rel. int. (%)): 219 ([M]$^+$, 28), 128 ([$C_{10}H_8$]$^+$, 100), 115 ([C_9H_7]$^+$, 54); Analysis calcd for $C_{11}H_9NO_4$: C, 60.28; H, 4.14; N, 6.39. Found: C, 59.93; H, 4.02; N, 6.34.

7-Amino-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**1b**)

A mixture containing 7-nitro tricyclic lactone **7a** (3.00 g, 13.7 mmol) and tin (powdered, 17.7 g, 0.149 mol) in ethanol (63 mL) and 10*N* HCl (59 mL) was heated at reflux for 1 h. After cooling, ethanol was removed *in vacuo*. The pH was adjusted to pH 8 by addition of saturated $NaHCO_3$ and insoluble tin salts were removed by filtration over hyflo. Extraction with ethyl acetate (3x), drying ($MgSO_4$) and removal of the solvent *in vacuo* afforded crude **1b** as a brownish solid (2.56 g, 99%). Recrystallization from ethyl acetate provided analytically pure **1b** (1.99 g, 77%) as pale yellow crystals.

Mp 128-130^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.22 (dd, 1H, ^{2}J 17.9 Hz, J 5.5 Hz, H_3), 2.54-3.05 (m, 4H, H_3, H_4 and H_{3a}), 3.70 (br s, 2H, NH_2), 5.66 (d, 1H, J 6.8 Hz, H_{8b}), 6.55 (dd, 1H, ^{4}J 2.1 Hz, J 7.9 Hz, H_6), 6.63 (d, 1H, ^{4}J 2.1Hz, H_8), 6.91 (d, 1H, J 7.9 Hz, H_5) ppm; MS (EI, m/z, rel. int. (%)): 189 ([M]$^+$, 100), 144 ([$C_{10}H_{10}N$]$^+$, 62), 130 ([C_9H_8N]$^+$, 34), 115 ([C_9H_7]$^+$, 10); Analysis calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.47; H, 5.81; N, 7.30.

5-Dimethylamino-naphtalene-1-sulfonic acid (2-oxo-3,3a,4,8b-tetrahydro-2H-indeno-[1,2-b]furan-7-yl)-amide (**8**)

To a solution of 7-amino tricyclic lactone **1b** (872 mg, 4.61 mmol) in pyridine (25 mL) was added dansyl chloride (purchased from Aldrich, 1.24 g, 4.61 mmol). The solution was stirred for 18 h with protection from light. Pyridine was removed *in vacuo* and the residue was dissolved in ethyl acetate and 1% HCl. The aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to give dansyl tricyclic lactone **8** (1.75 g, 90%) as a yellow solid. An analytically pure sample was obtained by crystallization from dichloromethane/acetic acid.

Mp 238-241^{0}C; ^{1}H-NMR (DMSO-d_6, 100 MHz): δ 2.20 (dd, 1H, ^{2}J 17.9 Hz, J 5.5 Hz, H_3), 2.43-3.29 (m, 4H, H_3, H_4 and H_{3a}), 2.72 (s, 6H, $N(CH_3)_2$), 5.64 (d, 1H, J 6.9 Hz), 7.00 (m, 2H, Ph), 7.17 (d, 1H, J 7.4 Hz, Ph), 7.55 (m, 2H, Ph), 8.14 (d, 1H, J 6.9 Hz, Ph), 8.29 (d, 1H, J 7.8 Hz, Ph), 8.37 (d, 1H, J 7.1 Hz, Ph), 10.8 (very br s, 1H, NH); MS (EI, m/z, rel. int. (%)): 422 ([M]$^+$, 100), 363 ([$C_{21}H_{19}N_2O_2S$]$^+$, 13), 170 ([$C_{12}H_{12}N$]$^+$, 57); Analysis calcd for $C_{23}H_{21}N_2O_4S$: C, 65.54; H, 5.02; N, 6.65, S, 7.61. Found: C, 65.21; H, 5.13; N, 6.57; S, 7.40.

7-Azido-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**9**)

To a solution of 7-amino tricyclic lactone **1b** (100 mg, 0.529 mmol) in TFA (10 mL) was added sodium nitrite (73 mg, 1.1 mmol) at 0^{0}C with protection from light. After 30 min sodium azide (344 mg, 5.29 mmol) was gradually added. After 15 min ethyl acetate (10mL) was added and

stirring was continued for 1h. TFA was removed *in vacuo* and the residue was dissolved in ethyl acetate and water. The aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were dried ($MgSO_4$), and concentrated *in vacuo* . The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) to afford 7-azido tricyclic lactone **9** (101 mg, 89%) as a yellow solid.
Mp 75-77^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.36 (dd, 1H, ^{2}J 18.0 Hz, J 5.5 Hz, H_3), 2.78-3.60 (m, 4H, H_3, H_4 and H_{3a}), 5.65 (d, 1H, J 7.0 Hz, H_{8b}), 6.99 (dd, 1H, ^{4}J 2.1 Hz, J 8.1 Hz, H_6), 7.14 (d, 1H, ^{4}J 2.1Hz, H_8), 7.25 (d, 1H, J 8.1 Hz, H_5) ppm; IR (CCl_4) ν 2120 (N_3), 1790 (C=O) cm^{-1}; MS (EI, m/z, rel. int. (%)): 215 ([M]$^+$, 23), 187 ([$C_{11}H_9NO_2$]$^+$, 100), 130 ([C_9H_8N]$^+$, 20), 115 ([C_9H_7]$^+$, 12); HRMS/EI: m/z calcd for $C_{11}H_9N_3O_2$: 215.0695. Found: 215.0694 ± 0.0010 amu.

7-Iodo-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**10a**) and *5-iodo-3,3a,4,8b-tetrahydro-indeno[1,2-b]furan-2-one* (**10b**)
To a solution of tricyclic lactone **6** (100mg, 0.575 mmol) and iodine (146 mg, 0.575 mmol) in tetrachloromethane (15 mL) was added bis-trifluoroacetoxy-iodobenzene (272 mg, 0.633 mmol) with stirring at room temperature. After 48 h the solvent was removed *in vacuo*. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 6:1) and collection of the pure fractions afforded 7-iodo tricyclic lactone **10a** (65 mg, 38%) and 5-iodo tricyclic lactone **10b** (48 mg, 28%) both as white solids. Analytical samples of **10a** and **10b** were obtained by recrystallization from dichloromethane/di*iso*propyl ether.
10a Mp 110-113^{0}C; R_f 0.30 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.34 (dd, 1H, ^{2}J 18.0 Hz, J 5.4 Hz, H_3), 2.76-3.55 (m, 4H, H_3, H_4 and H_{3a}), 5.83 (d, 1H, J 6.9 Hz, H_{8b}), 7.03 (d, 1H, J 8.0 Hz, H_5), 7.65 (dd, 1H, ^{4}J 1.6 Hz, J 8.0 Hz, H_6), 7.80 (br s, 1H, H_8) ppm; GC-MS (EI, m/z, rel. int. (%)): 300 ([M]$^+$, 20), 256 ([$C_{10}H_9I$]$^+$, 20), 129 ([$C_{10}H_9$]$^+$, 100), 115 ([C_9H_7]$^+$, 18); HRMS/EI: m/z calcd for $C_{11}H_9IO_2$: 299.9649 Found: 299.9645 ± 0.0010 amu.
10b Mp 165-167.5^{0}C; R_f 0.40 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.42 (dd, 1H, ^{2}J 18.0 Hz, J 5.1 Hz, H_3), 2.76-3.38 (m, 4H, H_3, H_4 and H_{3a}), 5.99 (d, 1H, J 6.9 Hz, H_{8b}), 7.02 (t, 1H, J 7.6 Hz, H_7), 7.45 (d, 1H, J 7.6 Hz, H_8), 7.74 (d, 1H, J 7.6 Hz, H_6) ppm; GC-MS (EI, m/z, rel. int. (%)): 300 ([M]$^+$, 9), 256 ([$C_{10}H_9I$]$^+$, 19), 129 ([$C_{10}H_9$]$^+$, 100), 115 ([C_9H_7]$^+$, 15); HRMS/EI: m/z calcd for $C_{11}H_9IO_2$: 299.9649 Found: 299.9648 ± 0.0010 amu.

7-Nitro-3(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,8b-tetrahydroindeno-[1,2-b]furan-2-one (**13a**)
Potassium *tert*-butoxide (563 mg, 5.03 mmol) was added in small quantities to a solution of 7-nitro tricyclic lactone **7a** (1.00 g, 4.57 mmol) and methyl formate (0.84 mL, 13.7 mmol) in THF (35 mL) with stirring at -78^{0}C under nitrogen. The mixture was allowed to warm to room temperature and stirred for 18 h. Then THF was removed *in vacuo* and the residue was dissolved in DMF (35 mL). The mixture was cooled to -60^{0}C, and bromo butenolide **11** (0.97 g, 5.5. mmol) in DMF (5 mL) was gradually added under nitrogen. The mixture was brought to room temperature and stirred for 18 h. Work-up was accomplished by addition of acetic acid (0.60 g, 10 mmol) followed by concentration of the suspension *in vacuo*. The residue was dissolved in chloroform and water. The aqueous phase was extracted with chloroform (2x). The combined organic layers were washed with water (1x), dried ($MgSO_4$), and concentrated *in vacuo* . The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) and afforded two separated diastereomers of **13a** (549 mg, 35%) with R_f 0.12 and 0.18 (hexane/ethyl

acetate 1:1) as pale yellow solids. Only the fast moving diastereomer could be obtained in an analytically pure form by recrystallization from ethyl acetate.
Mp 216-219^{0}C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.06 (m, 3H, CH_3), 3.21 (dd, 1H, ^{2}J 18.0 Hz, J 3.2 Hz, H_4), 3.52 (dd, 1H, ^{2}J 18.0 Hz, J 9.3 Hz, H_4), 4.08 (m, 1H, H_{3a}), 5.99 (d, 1H, J 8.0 Hz, H_{8b}), 6.20 (m, 1H, OCHO), 6.97 (m, 1H, =CH), 7.39 (d, 1H, J 8.4 Hz, H_5), 7.51 (d, 1H, ^{4}J 2.3 Hz, =CHO), 8.23 (dd, 1H, ^{4}J 1.9 Hz, J 8.4 Hz, H_6), 8.37 (d, 1H, ^{4}J 1.9 Hz, H_8) ppm; MS (EI, m/z, rel. int. (%)): 343 ($[M]^+$, 1), 247 ($[C_{12}H_9NO_5]^+$, 2), 219 ($[C_{11}H_9NO_4]^+$, 1), 97 ($[C_5H_5O_2]^+$, 100); Analysis calcd for $C_{17}H_{13}NO_7$: C, 59.48; H, 3.82; N, 4.08. Found: C, 59.21; H, 3.81; N, 4.11.
7-Nitro GR24 **13a** could be prepared in alternative manner by nitration of GR24 by the procedure described for the synthesis of **7a**. Starting from GR24 (100 mg, 0.336 mmol) **13a** was obtained in 92% yield. ^{1}H-NMR data were in complete agreement with those reported above.

7-Iodo-3(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,8b-tetrahydroindeno-[1,2-b]furan-2-one (**15**)
Potassium *tert*-butoxide (167 mg, 1.64 mmol) was added in small quantities to a solution of 7-iodo tricyclic lactone **10a** (447 mg, 1.49 mmol) and ethyl formate (1.20 mL, 14.9 mmol) in THF (25 mL) with stirring at 0^{0}C under nitrogen. The mixture was allowed to warm to room temperature and stirred for 18 h. Then excess acetic acid (1 mL) was added and the solvent was removed *in vacuo*. The mixture was dissolved in ethyl acetate and saturated NH_4Cl. The aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were dried ($MgSO_4$), and concentrated *in vacuo* to give crude formyl tricyclic lactone as a pale yellow solid. Starting **10a** was removed by washing with diethyl ether (2x) to provide pure hydroxymethyleno lactone in 50% yield.
^{1}H-NMR (acetone-d_6, 100 MHz): δ 2.85-3.33 (m, 2H, H_4), 3.82 (m, 1H, H_{3a}), 5.69 (d, 1H, J 7.5 Hz, H_{8b}), 6.93 (d, 1H, J 7.7 Hz, H_5), 7.38-7.61 (m, 3H, H_6, H_8, =CHO), 9.6 (br s, 1H, OH).
To a solution of thus obtained hydroxymethyleno lactone (150 mg, 0.457 mmol) in DMF (10 mL) was added potassium *tert*-butoxide (56.0 mg, 0.503 mmol) at 0^{0}C in a nitrogen atmosphere. The mixture was cooled to -60^{0}C and bromo butenolide **11** (97.0 mg, 0.548 mmol) in DMF (2 mL) was gradually added. Work-up was accomplished as described for the preparation of 7-nitro GR24 **13a** (*vide supra*). Purification by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) afforded two separated diastereomers in 57% yield. The fast moving diastereomer of **15** (R_f 0.35, hexane/ethyl acetate 1:1) was crystallized from ethyl acetate to give **15** as colorless needles.
Mp 206-209^{0}C; ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.04 (m, 3H, CH_3), 3.03 (dd, 1H, ^{2}J 17.0 Hz, J 3.5 Hz, H_4), 3.39 (dd, 1H, ^{2}J 17.0 Hz, J 8.8 Hz, H_4), 3.94 (m, 1H, H_{3a}), 5.90 (d, 1H, J 7.8 Hz, H_{8b}), 6.19 (m, 1H, OCHO), 6.95 (m, 1H, =CH), 6.97 (d, 1H, J 8.0 Hz, H_5), 7.48 (d, 1H, ^{4}J 2.5 Hz, =CHO), 7.64 (dd, 1H, ^{4}J 1.6 Hz, J 8.0 Hz, H_6), 7.83 (d, 1H, ^{4}J 1.6 Hz, H_8) ppm; MS (EI, m/z, rel. int. (%)): 424 ($[M]^+$, 4), 327 ($[C_{12}H_8O_3I]^+$, 26), 97 ($[C_5H_5O_2]^+$, 100); HRMS/EI: m/z calcd for $C_{17}H_{13}IO_5$: 423.9804. Found: 423.9810.
The slow moving diastereomer of **15** (R_f 0.26, hexane/ethyl acetate 1:1) was recrystallized from hexane/ethyl acetate to give colorless crystals.
Mp 189-191^{0}C; ^{1}H-NMR data are the same as for the fast moving diastereomer of **15** (*vide supra*); MS (EI, m/z, rel. int. (%)): 424 ($[M]^+$, 6), 327 ($[C_{12}H_8O_3I]^+$, 34), 97 ($[C_5H_5O_2]^+$, 100); HRMS/EI: m/z calcd for $C_{17}H_{13}IO_5$: 423.9804. Found: 423.9810 ± 0.0010 amu.

7-Amino-3(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,8b-tetrahydroindeno-[1,2-b]furan-2-one (**16**)

A solution of amino tricyclic lactone **1b** (440 mg, 2.33 mmol) and benzaldehyde (247 mg, 2.33 mmol) in ethyl acetate (20 mL), in the presence of mol. sieves 4Å, was stirred for 12 h. at room temperature Then $MgSO_4$ was added and the mixture was filtered over hyflo. The solvent was removed *in vacuo* to give imine **17** (645 mg, 100%) as a pale yellow solid, which was used immediately in the coupling reaction.

^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.36 (dd, 1H, ^{2}J 18.1 Hz, J 5.4 Hz, H_3), 2.70-3.56 (m, 4H, H_3, H_4 and H_{3a}), 5 87 (d, 1H, J 6 8 Hz, H_{8b}), 7.24-7.27 (m, 3H, Ph), 7.43-7.53 (m, 3H, Ph), 7.64-7.94 (m, 2H, Ph), 8.44 (s, 1H, N=CH) ppm.

To a solution of freshly prepared imine **17** (645 mg, 2.33 mmol) and methyl formate (0.5 mL, 7 mmol) in THF (25 mL) was added potassium *tert*-butoxide (287 mg, 2.56 mmol) in small portions at 0^{0}C under nitrogen. The mixture was allowed to warm to room temperature and stirred for 18 h. Then THF was removed *in vacuo* and the residue was dissolved in DMF (20 mL). The mixture was cooled to -60^{0}C, and bromo butenolide **11** (494 mg, 2.70 mmol) in DMF (3 mL) was gradually added under nitrogen. The mixture was brought to room temperature and stirred for 18 h. Work-up was accomplished by removing the solvent *in vacuo* The residue was dissolved in dichloromethane and saturated $NaHCO_3$. The aqueous phase was extracted with dichloromethane (2x) The combined organic layers were washed with saturated $NaHCO_3$ (1x), dried ($MgSO_4$), and concentrated *in vacuo* to provide crude 7-benzal-imino GR24 in 78% yield. After flash chromatography (SiO_2, dichloromethane, followed by ethyl acetate/dichloromethane 3:1) 7-amino GR24 **16** (190 mg, 27%) was obtained as a mixture of two diastereomers, which could not be separated Crystallization from *n*-butyl acetate afforded **16** as pale yellow crystals

^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.94 (m, 3H, CH_3), 2.86 (dd, 1H, ^{2}J 16.3 Hz, J 3.0 Hz, H_4), 3.23 (dd, 1H, ^{2}J 16.3 Hz, J 8.8 Hz, H_4), 3 75 (m, 3H, NH_2 and H_{3a}), 5 77 (d, 1H, J 7 8 Hz, H_{8b}), 6.10 (m, 1H, OCHO), 6 59 (dd, 1H, ^{4}J 2 2 Hz, J 8.0 Hz, H_6), 6.70 (d, 1H, ^{4}J 2.2 Hz, H_8), 6.88 (m, 1H, =CH), 6 92 (d, 1H, J 8 0 Hz, H_5), 7.39 (d, 1H, ^{4}J 2.5 Hz, =CHO) ppm; MS (EI, m/z, rel int. (%)): 313 ([M]$^+$, 57), 216 ([$C_{12}H_{10}NO_3$]$^+$, 40), 188 ([$C_{11}H_{10}NO_2$]$^+$, 3), 97 ([$C_5H_5O_2$]$^+$, 100), Analysis calcd for $C_{17}H_{15}NO_5$. C, 65.17, H, 4.82; N, 4.47 Found: C, 65.16, H, 4.96; N, 4.31.

7-Azido-3(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,8b-tetrahydroindeno-[1,2-b]furan-2-one (**14**)

Sodium nitrite (33 mg, 0.48 mmol) was added to a solution of a mixture of diastereomers of 7-amino GR24 **16** (138 mg, 0.44 mmol) in 80% acetic acid (20 mL) at 0^{0}C with protection from light. After 5 min sodium azide (32 mg, 0.48 mmol) was added and stirring was continued for 2h Work-up was accomplished by concentration of the solution *in vacuo* The residue was dissolved in ethyl acetate and saturated $NaHCO_3$. The aqueous phase was extracted with ethyl acetate (2x) The combined organic layers were washed with saturated $NaHCO_3$ (1x), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 1·1) afforded two separated diastereomers of **14** (131 mg, 88%) as white solids.

Fast moving diastereomer of **14** : Mp 151-154^{0}C; R_f 0.29 (hexane/ethyl acetate 1.1); ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 2.04 (m, 3H, CH_3), 3.06 (dd, 1H, ^{2}J 16.7 Hz, J 3.6 Hz, H_4), 3 41 (dd, 1H, ^{2}J 16.7 Hz, J 8 8 Hz, H_4), 3.96 (m, 1H, H_{3a}), 5.91 (d, 1H, J 7.8 Hz, H_{8b}), 6.19 (m, 1H, OCHO), 6.93-7.25 (m, 4H, Ph and =CH), 7 48 (d, 1H, ^{4}J 2.5 Hz, =CHO) ppm; IR (CCl_4) ν 2120 (N_3), 1795 (C=O), 1765 (C=O), 1685 (C=C, enol ether); MS (EI, m/z, rel. int. (%)): 339 ([M]$^+$, 2), 313

($[C_{17}H_{15}NO_5]^+$, 1), 214 ($[C_{12}H_8NO_3]^+$, 2), 97 ($[C_5H_5O_2]^+$, 100); HRMS/EI: m/z calcd for $C_{17}H_{13}N_3O_5$: 339.0855 Found: 339.0856 ± 0.0013 amu.
Slow moving diastereomer of **14** : Mp 164-167^{0}C; R_f 0.19 (hexane/ethyl acetate 1:1); ^{1}H-NMR ($CDCl_3$, 100 MHz) data are the same as described for the fast moving diastereomer of **14**; MS (EI, m/z, rel. int. (%)): 339 ($[M]^+$, 2), 311 ($[C_{17}H_{13}NO_5]^+$, 3), 215 ($[C_{12}H_9NO_3]^+$, 3), 97 ($[C_5H_5O_2]^+$, 100); HRMS/EI: m/z calcd for $C_{17}H_{13}N_3O_5$: 339.0855 Found: 339.0856 ± 0.0013 amu.

5-Dimethylamino-naphtalene-1-sulfonic acid-[3-(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy-methylene)-2-oxo-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-7-yl]-amide (**12**)
A mixture of slow and fast moving 7-amino GR24 **16** (170 mg, 0.543 mmol) and dansyl chloride (146 mg, 0.543 mmol) were dissolved in pyridine (10 mL). The solution was stirred 65 h with protection from light. Then, the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate and 5% oxalic acid. The aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were washed with 5% oxalic acid (1x), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) to provide two separated diastereomers of **12** (264 mg, 89%) as pale yellow solids with R_f 0.20 and 0.17 (hexane/ethyl acetate 1:1). An analytically pure sample of the fast moving diastereomer of **12** was obtained by recrystallization from di*iso*propyl ether/dichloromethane.
Mp 186-188^{0}C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.03 (m, 3H, CH_3 D-ring), 2.88 (s, 6H, $N(CH_3)_2$), 2.96 (dd, 1H, ^{2}J 16.4 Hz, J 3.5 Hz, H_4), 3.28 (dd, 1H, ^{2}J 16.4 Hz, J 9.4 Hz, H_4), 3.86 (m, 1H, H_{3a}), 5.72 (d, 1H, J 8.2 Hz, H_{8b}), 6.14 (m, 1H, OCHO), 6.81 (s, 1H, NH), 6.92 (m, 1H, =CH), 6.96 (s, 1H, H_8), 7.00 (s, 2H, H_5 and H_6), 7.19 (d, 1H, J 7.6 Hz, Ph dansyl), 7.42 (t, 1H, J 7.6 Hz, Ph dansyl), 7.43 (d, 1H, ^{4}J 2.4 Hz, =CHO), 7.59 (t, 1H, J 8.4 Hz, Ph dansyl), 8.14 (d, 1H, J 7.6 Hz, Ph dansyl), 8.31 (d, 1H, J 8.4 Hz, Ph dansyl), 8.50 (d, 1H, J 8.4 Hz, Ph dansyl), ppm; MS (CI, m/z, rel. int. (%)): 450 ($[M+1-C_5H_5O_2]^+$, 1), 422 ($[C_{23}H_{22}N_2O_4S]^+$, 3), 217 ($[C_{12}H_{11}NO_3]^+$, 11), 171 ($[C_{12}H_{13}N]^+$, 2), 97 ($[C_5H_5O_2]^+$, 12), 28 ($[CO]^+$, 100); Analysis calcd for $C_{29}H_{26}N_2O_7S$: C, 63.73; H, 4.79; N, 5.13; S, 5.83. Found: C, 63.50; H, 4.83; N, 5.10; S, 5.83; Fluorescence (c 2.0 mM, MeOH): $\lambda_{exc.}$ 350 nm (ε $4.12*10^3$), $\lambda_{em.}$ 525 nm.

N-[3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-2-oxo-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-7-yl]-methanesulfonamide (**18**)
The procedure described for the preparation of **12** was followed using amino GR24 **16** (100 mg, 0.320 mmol), mesyl chloride (36.6 mg, 0.320 mmol) and pyridine (10 mL). Work-up followed by purification by flash chromatography (SiO_2, dichloromethane/ethyl acetate 3:1) gave mesyl GR24 **18** (98 mg, 78%) as a mixture of diastereomers, which could not be separated. Recrystallization from ethanol afforded **14** as a white solid.
^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.05 (m, 3H, $=CH_3$), 2.97 (s, 3H, SCH_3), 3.05 (dd, 1H, ^{2}J 16.9 Hz, J 3.0 Hz, H_4), 3.39 (dd, 1H, ^{2}J 16.9 Hz, J 8.8 Hz, H_4), 3.96 (m, 1H, H_{3a}), 5.92 (d, 1H, J 7.8 Hz, H_{8b}), 6.21 (m, 1H, OCHO), 7.00 (m, 1H, =CH), 7.14-7.27 (m, 4H, Ph and NH), 7.54 (d, 1H, ^{4}J 2.5 Hz, =CHO) ppm; MS (EI, m/z, rel. int. (%)): 391 ($[M]^+$, 1), 295 ($[C_{13}H_{13}NO_5S]^+$, 3), 216 ($[C_{12}H_{10}NO_3]^+$, 1), 97 ($[C_5H_5O_2]^+$, 12), 28 ($[CO]^+$, 100); Analysis calcd for $C_{18}H_{17}NO_7S$: C, 55.24; H, 4.38; N, 3.58; S, 8.19. Found: C, 54.89; H, 4.24; N, 3.72; S, 8.08.

Biological activity

Seeds

Seeds of *Striga hermonthica* and *Orobanche crenata* were harvested in Sudan in 1987 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of test solutions

A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0.1, 0.01 and 0.001 mg/L test compound and 0.1, 0.01, 0.001 and 0.0001% (v/v) acetone, respectively.

Bioassays

For surface sterilization seeds of *Striga hermonthica* and *Orobanche crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter, approximately 30-70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20^{0}C for *Orobanche* seeds and at 30^{0}C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at the indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat

In each test series aqueous solutions with 0.1, 0.01, 0 001 and 0.0001% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (concentrations of 1, 0.1, 0.01 and 0.001 mg/L) were used as positive controls. All tests were performed in duplicates, and in each test the germination percentages were determined on 12 disks.

For full details of the bioassay, see ref.[27]

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Chapter 13

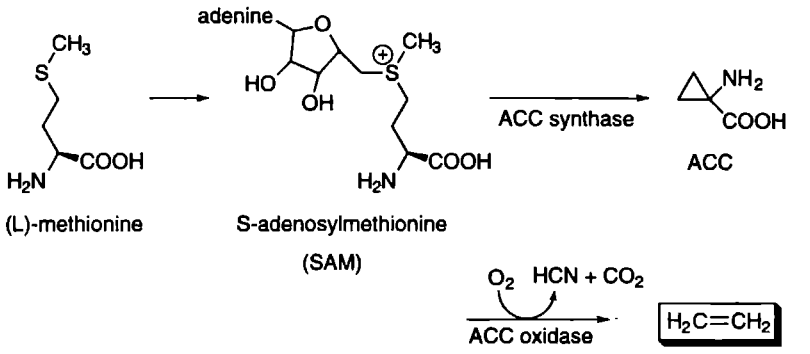
Synthesis of Vinyl Glycine Derivatives, Analogs of Aminoethoxyvinyl Glycine (AVG)

Abstract: A formal synthesis of some protected γ-phenylsulfanyl- and γ-methylsulfanyl vinyl glycine derivatives, which are analogs of aminoethoxyvinyl glycine (AVG), has been achieved. The synthesis of these potential inhibitors of the biosynthesis of ethene starts either from (L)-methionine using a Pummerer reaction, or from a cyclic orthoester protected (L)-serinal synthon, employing a Peterson olefination reaction.

13.1 Introduction

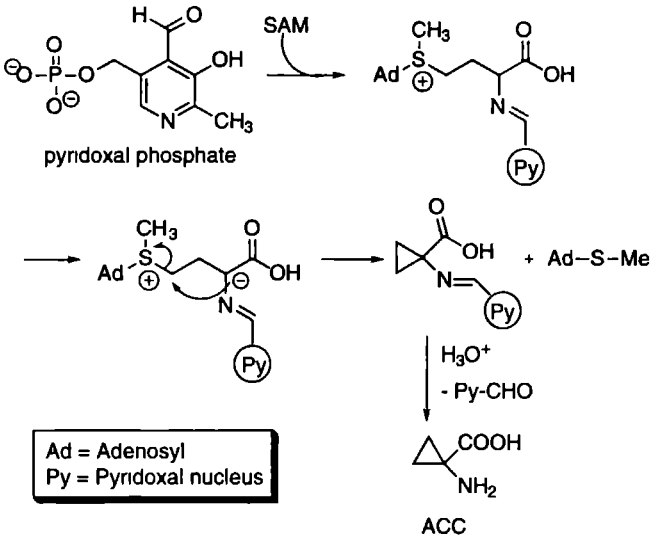
Inhibition of the germination of seeds of the parasitic weed *Striga hermonthica* can at least in principle be achieved by blocking the biosynthesis of ethene. The role of ethene in the germination of seeds of *Striga hermonthica* is discussed in chapter 14. The rate-limiting step in the biosynthesis of ethene, *viz.* the formation of ACC from SAM (fig 1), is strongly inhibited by β,γ-unsaturated glycine derivatives.[1a-c]

Scheme 1

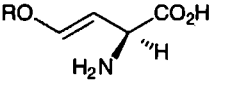


These compounds are well established inhibitors of pyridoxal phosphate-dependent enzymes in general.[2] ACC-synthase is such an enzyme, which requires pyridoxal phosphate as a co-factor. The formation of ACC from SAM is mediated by pyridoxal phosphate as depicted in scheme 2.[1a,3]

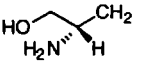
Scheme 2

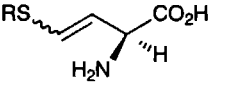


The role of the coenzyme is the formation of a Schiff base, thereby facilitating the abstraction of an α-H, which gives rise to a γ-elimination reaction to produce ACC. The mechanism of specific *inhibition* of pyridoxal phosphate dependent enzymes by β,γ-unsaturated glycines is also based on the formation of a Schiff base. In principle, a relatively unreactive species is transformed to a reactive intermediate nearby the active site, leading to (ir)reversible inactivation of the enzyme. For this reason these specific inhibitors are commonly referred to as suicide enzyme inactivators[2] or k_{cat} inhibitors.[3] An example of this concept is the inhibition of aspartate transaminase by the action of *trans*- γ-methoxyvinyl glycine (MVG) as is depicted in scheme 3.[4]



MVG, R = Me
AVG, R = $H_2NCH_2CH_2$

Rhizobitoxine, R = 

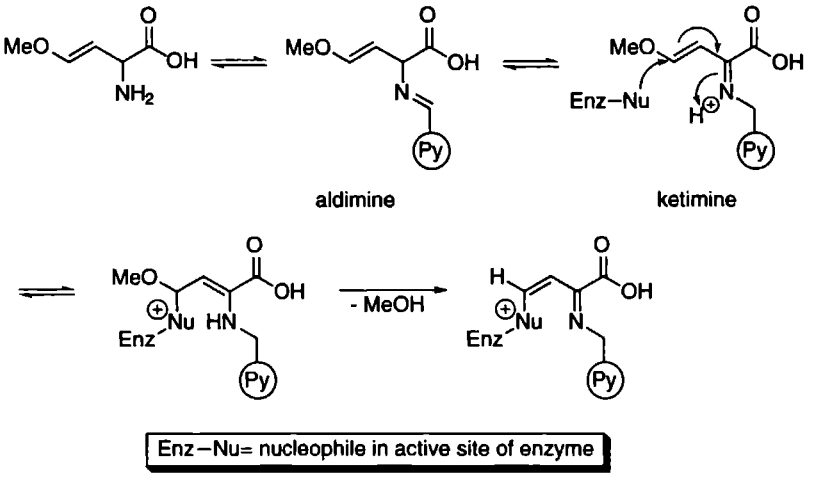


(*E*)-**1** and (*Z*)-**1**, R = Me
(*E*)-**2** and (*Z*)-**2**, R = Ph

The activation is the result of an aldimine-ketimine tautomerization, which enables the addition of a nucleophilic species in the active site of the enzyme in a Michael fashion Potent k_{cat} inhibitors are (L)-γ-alkoxyvinyl glycines, *e.g.* methyl enol ether MVG (from *Pseudomonas aeruginosa)*, aminoethyl enol ether AVG (from *Streptomyces*) and rhizobitoxine (from

Rhizobium japonicum), which were all isolated from natural sources and have interesting biological properties.[5]

Scheme 3



These compounds strongly inhibit ethene production by plant tissue through inactivation of the pyridoxal phosphate linked enzyme ACC-synthase. Total syntheses of these natural products, in some cases in a protected form, have been reported (MVG, refs.[5-10]; AVG, ref.[11]; rhizobitoxine, ref.[6]). However, these syntheses are inefficient multi-step processes. Moreover, as most syntheses involve a double bond migration from the α,β-position to the desired β,γ-position, racemic compounds were obtained. To obtain optically active inhibitors a subsequent enzymatic resolution was required. So far, scarce attention has been paid to synthetic inhibitor analogues. Mechanistically, only vinyl glycine derivatives with modifications in the alkoxy moiety are allowed. Recently, the preparation of some γ-aryloxy vinyl glycine analogues,[10] methylthio ethers (L)-*Z*-**1**, (L)-*E*-**1**[12] and phenylthio ethers (DL)-*Z*-**2**, (DL)-*E*-**2**[13] has been reported. However, the yields and selectivities were very poor and no data on their biological activities were given.

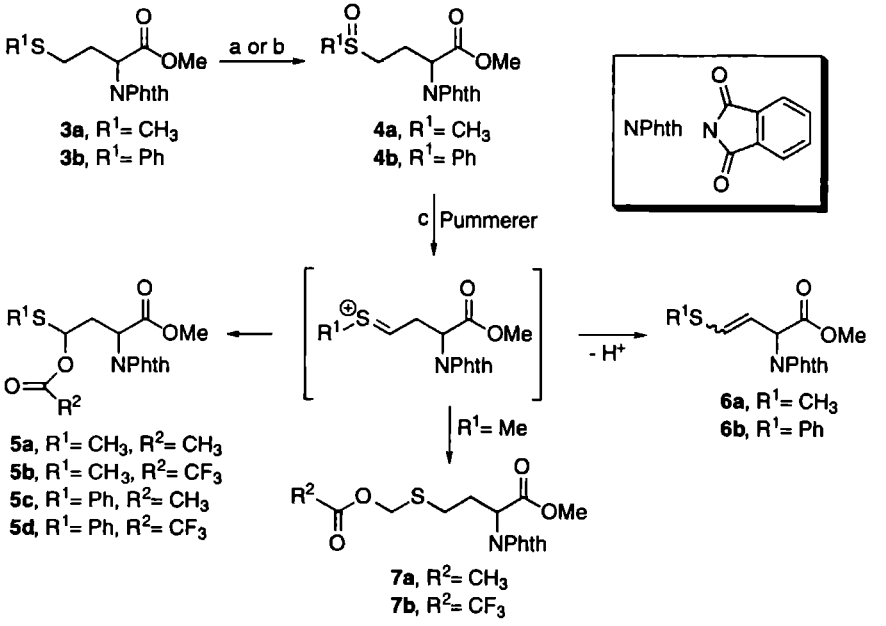
In this chapter preliminary results are described of the preparation of vinyl glycines, substituted with various alkylsulfanyl and arylsulfanyl groups. These compounds may have interesting biological properties such as inhibition of the ethene biosynthesis. Consequently, they may show an inhibitory effect on the germination of *Striga* seeds.

13.2 Results and discussion

The main problem in the synthesis of this type of anomalous α-amino acids is the introduction of the labile enolthio ether function in molecules containing a reactive amino acid

unit. Other important aspects are stereocontrol and control of the double bond geometry, which is important because *Z*- and *E*-isomers may exert different biological effects, as was demonstrated by a comparison of (L)-*trans*-MVG and (L)-*cis*-MVG.[14] Two synthetic approaches using the chiral pool were considered, *viz.* one starting from (L)-methionine and one from (L)-serine, as outlined in schemes 4 and 6, respectively

Scheme 4



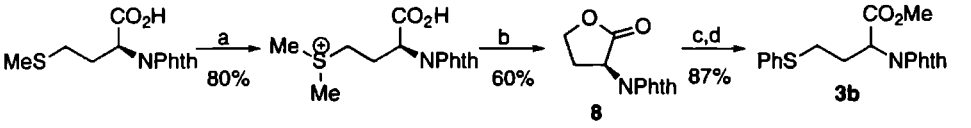
a (R^1= Me), $NaIO_4$ in MeOH/ H_2O, c y 96%, b (R^1= Ph), *m*CPBA, c y 95%

c acetic anhydride (Ac_2O) or trifluoroacetic anhydride (TFAA)

The key step in the first approach (scheme 4) is a Pummerer reaction of methionine derived sulfoxides **4** in the presence of an appropiate anhydride [15] *N*-Phthaloyl protected (L)-methionine methyl ester **3a** was obtained by standard procedures [16] The subsequent oxidation was performed employing sodium metaperiodate ($NaIO_4$) in methanol/water in 96% yield (*cf* oxidation of *N*-benzyloxycarbonyl-methionine methyl ester).[17]

Phenyl derivative **3b** was prepared by a modified literature procedure, which is depicted in scheme 5 [13] The ring opening of lactone **8** was considerably improved by using 1 1 equiv of thiophenoxide instead of 2.2 equiv as reported in the literature [13] Oxidation with $NaIO_4$ was not successful due to the poor solubility of **3b** in methanol-water However, the use of *meta*-chloroperbenzoic acid (*m*CPBA) in dichloromethane gave the sulfoxide **4b** in 95% yield

Scheme 5



a) MeI; b) KO^{t}Bu in DMF; c) PhSH, KO^{t}Bu (1.1 equiv.), HMPA in THF; d) MeOH, H_2SO_4

Both substrates were subjected to a Pummerer reaction (scheme 4) using acetic anhydride (Ac_2O) or trifluoroacetic anhydride (TFAA), in some cases in the presence of an acid catalyst. The results are collated in Table 1. It should be emphasized that the characterization of compounds **4a,b**, **5a-d** and **7a,b**, which is based on the ^{1}H-NMR spectra, is tentative. The structures of the *E*- and *Z*-isomers of **6a** and **6b** were unambigiously assigned by comparison of ^{1}H-NMR data with published values.[13]

Table 1. Pummerer reaction of sulfoxides **4a,b** under various conditions

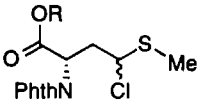
					product ratio (%)[a]			
entry	substrate	El.[b]	catalyst	rx. time	**5**	**7**	**6**	*E*:*Z*[c]
1	**4a**	Ac_2O[d]	none	72h[e]	48 (**a**)	52 (**a**)	0	
2	**4a**	Ac_2O[d]	TsOH, 10%	18h[g]	0	20 (**a**)	80 (**a**)	2:1
3	**4a**	TFAA[e]	none	0.5h	0	60 (**b**)	40 (**a**)	7:1
4	**4b**	Ac_2O[f]	AcOH	72h	46 (**c**)		54 (**b**)	6:1
5	**4b**	Ac_2O[d]	TsOH, 1%	8h[g]	75 (**c**)		25 (**b**)	3:1
6	**4b**	Ac_2O[d]	TsOH, 10%	18h[h]	20 (**c**)		80 (**b**)	5:1
7	**4b**	TFAA[e]	none	0.5h	54 (**d**)		46 (**b**)	5:1

a) Determined by ^{1}H-NMR analysis of the crude reaction mixture
b) Electrophilic species
c) Ratio of *E*- and *Z*-isomers of vinyl sulfides **6a,b**
d) Used as the solvent
e) 1.5 Equivalent, solvent CH_2Cl_2, room temperature
f) Reaction performed in a 1:1 mixture of Ac_2O and AcOH at 115^{0}C
g) Reaction performed at 80^{0}C
h) Reaction performed at 90^{0}C

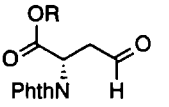
The data in Table 1 reveal that the reaction conditions are crucial for the product distribution. When sulfoxide **4a** was subjected to the Pummerer reaction, using Ac_2O as the solvent in the absence of an acid catalyst, two regioisomeric acetates **5a** and **7a** were obtained (entry 1), which could not be separated chromatographically. The product distribution changes dramatically in the presence of an acid catalyst (entries 2,4,5,6). Under these conditions abstraction of the β-proton (scheme 4) to give the corresponding vinylthio ethers **6a,b** in an *E*-selective fashion, plays a competitive role. The possibility that **6a,b** were formed as a result of elimination of acetic acid from the intermediates **5a,c** was excluded, since exposure of these

thioacetals to the reaction conditions did not result in the formation of **6a,b**. Interestingly, even the amount of acid catalyst is critical for the outcome of the reaction (*cf.* entries 5 and 6). The use of TFAA as the electrophilic species in stoichiometric amounts decreased the reaction time considerably (15-30 min) and was beneficial for the *E*/*Z*-ratio (entries 3 and 7). Chromatographic separation of vinyl sulfides **6**, and acetals **5** and **7** could readily be achieved. It was not possible to obtain the *E*- and *Z*-isomers of **6** separately.

A similar Pummerer-type reaction, *viz* chlorination of **3a,b** with either sulfuryl chloride[16] or *N*-chlorosuccinimide[13] has recently been reported. In the first case, the resulting highly unstable halo compound **9a** was hydrolyzed directly to homoserinal derivative **10a**. The use of NCS caused the formation of many products, such as **9b**, **10b**, *E*-**6a** and *Z*-**6a**, with poor selectivity.



9a, R = Et
9b, R = Me

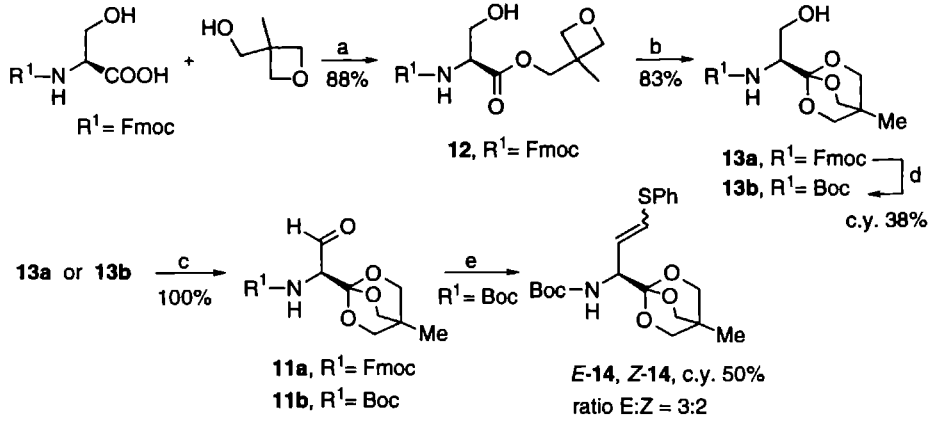


10a, R = Et
10b, R = Me

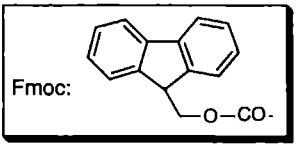
In conclusion, the present route starting from (L)-methionine using a Pummerer reaction in the presence of an acid catalyst gives relatively simple access to methyl and phenyl vinyl sulfides **6a** and **6b** in an *E*-selective fashion. This approach is far more convenient and selective in comparison with previously reported syntheses.

The second approach (scheme 6) is based on a recent report on the synthesis of the novel chiral (L)-serine aldehyde equivalent **11a**.[18] A base-stable cyclic orthoester was used as carboxyl protection. The essence of this protection strategy is that the acidity of the α-proton is decreased, resulting in a decreased tendency to enolize and hence to racemize. Moreover, the less acidic α-proton allows addition of a wide variety of nucleophiles to the aldehyde function.[18] Introduction of the (thio)enol moiety involves a one carbon homologation and may be achieved by addition of a suitable formyl anion equivalent, such as **15-18**, followed by elimination. Cyclic orthoester protected (L)-serinal **11a**, bearing an Fmoc amino protection group, was prepared in two steps via the oxetane ester **12** as depicted in scheme 6.[18] The use of this bridged orthoester as carboxylic acid protection has been introduced by Corey and Raju,[19] and has found hitherto only limited applications in amino acid chemistry.[20,21]

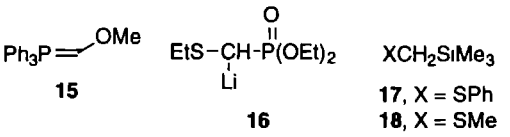
Scheme 6



a) DCC, DMAP; b) $BF_3.Et_2O$; c) oxalyl chloride, DMSO, *i*-Pr_2EtN
d) 1. piperidine, MeOH, 2. Boc_2O, DMAP; e) **17**, *n*-BuLi



It was claimed that, despite the presence of the base labile Fmoc protective group, a variety of addition reactions could be performed on the aldehyde function in **11a**, including Grignard, Reformatsky and Wittig reactions.[18] However, in our hands the use of the unstabilized ylid **15** or the lithio phosphonate **16**[22] did not lead to the expected addition product. Therefore, the Fmoc group was replaced by the base-stable *tert.*-butyloxycarbonyl (Boc) group to give **13b** in a moderate overall yield of 38% (not optimized). The desired serinal synthon **11b** was obtained from **13b** in quantitative yield by a Swern oxidation. A Peterson-type olefination reaction, using phenylthiomethyltrimethylsilane **17**,[23] provided *E*- and *Z*-vinyl sulfides **14** in a 3:2 ratio, which could be separated by flash chromatography. *E/Z*-Assignments were based on the ^3J-coupling constants of the olefinic protons, which amount to 9.6 Hz and 15.2 Hz for the *Z*- and the *E*-isomers, respectively.



This route thus provides access to the formal synthesis of a γ-substituted vinyl glycine derivative. So far, the deprotection of the amino function and the cyclic orthoester has not been achieved.

13.3 Experimental

General remarks
100 MHz ^{1}H-NMR spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard). All coupling constants are given as ^3J in Hz, unless indicated otherwise IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer For mass spectra a double focussing VG7070E mass spectrometer was used GLC was conducted with a Hewlet-Packard HP 5890 gas chromatograph, using a capillary column (25m) of HP-1, and nitrogen (2 ml/min, 0.5 atm) as the carrier gas Melting points were measured with a Reichert Thermopan microscope and are uncorrected.
Solvents were dried using the following methods· Dichloromethane was distilled from P_2O_5 Hexane was distilled from CaH_2 Ethyl acetate was distilled from potassium carbonate Tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0 25 mm) using the eluents indicated Spots were visualized with UV or using a molybdate spray Flash-chromatography was carried out at a pressure of *ca.* 1 5 bar, using Merck Kieselgel 60H Column chromatography at atmospheric pressure was carried out, using Merck Kieselgel 60.

Methyl 2-(phthaloylimino)-4-phenylsulfanyl-butanoate (**3b**)
The preparation of **3b** was carried out following a modified literature procedure [13] To a suspension of potassium *tert*-butoxide (10 7 g, 95.1 mmol) in THF (225 mL) were added thiophenol (10 5 g, 95.1 mmol) and HMPA (16.6 mL, 1 0 eq.) Phthalimido lactone **8**[13] (20 0 g, 86 6 mmol) was added and the thus obtained suspension was heated under reflux for 3h The resulting solution was concentrated *in vacuo* and the residue was dissolved in a mixture of satd $NaHCO_3$ and ethyl acetate. The aqueous phase was washed with ethyl acetate (2x) and acidified with 5% H_2SO_4 to pH 2 until a white precipitate settled. Extraction with ethyl acetate (2x) and washing the combined organic layers with water (4x) and brine (1x), drying ($MgSO_4$) and concentration *in vacuo* afforded 2-(phthaloylimino)-4-phenylsulfanyl-butanoic acid as an oil, which was triturated by stirring in di*iso*propyl ether to give a white solid (25 8 g, 87%). Mp 133^{0}C, ^{1}H-NMR ($CDCl_3$, 100 MHz) δ 2 42-2 72 (m, 2H, $SCH_2C\underline{H}_2$), 2.78-3 10 (m, 2H, $SC\underline{H}_2CH_2$), 5 19 (t, 1H, J 7.4 Hz, C<u>H</u>N), 7.17-7.33 (m, 5H, SPh), 7 68-7 91 (m, 4H, phthaloyl), 11.52 (br s, 1H, $CO_2\underline{H}$) ppm.
The thus obtained carboxylic acid (1.00 g, 2.93 mmol) was dissolved in a mixture of benzene (50 mL) and methanol (2.4 mL, 59 mmol). Sulfuric acid (16 µL, 0.3 mmol) was added and the mixture was heated under reflux using a Dean-Stark trap. Every hour methanol (2 4 mL) was added, until the conversion was complete according to TLC analysis (8h). The solvent was removed *in vacuo* and the residue was dissolved in a mixture of ethyl acetate and satd $NaHCO_3$. The organic phase was washed with satd. $NaHCO_3$ (2x), water (1x) and brine (1x). Drying ($MgSO_4$) and concentration *in vacuo* afforded **3b** (1 03 g, 99%) as a colorless oil, which solified on standing. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2 41-2 64 (m, 2H, $SCH_2C\underline{H}_2$), 2 80-3 12 (m, 2H, $SC\underline{H}_2CH_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 5.14 (dd, 1H, J 6.7 Hz, J 8 1 Hz, C<u>H</u>N), 7.14-7 35 (m, 5H, SPh), 7 69-7 92 (m, 4H, phthaloyl) ppm.

Methyl 2-(phthaloylimino)-4-methylsulfinyl-butanoate (**4a**)
An aqueous solution (21 mL) of sodium metaperiodate (3.84 g, 19.0 mmol) was gradually added to a vigorously stirred ice-cold solution of *N*-phthaloyl-L-methionine methyl ester **3a** (5.02 g, 17.1 mmol) in methanol (50 mL). The reaction mixture was stirred for 18h at room temperature and the precipitated iodate was filtered off. The filtrate was extracted with chloroform (6x) and the combined organic extracts were washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give **4a** (5.08 g, 96%) as a colorless oil, which was sufficiently pure for further reactions. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.57 (s, 3H, S(O)C$\underline{H}_3$), 2.64-2.88 (m, 4H, C$\underline{H}_2$), 3.76 (s, 3H, OC$\underline{H}_3$), 4.97 (m, 1H, C$\underline{H}$N), 7.73-7.95 (m, 4H, phthaloyl) ppm.

Methyl 2-(phthaloylimino)-4-phenylsulfinyl-butanoate (**4b**)
A solution of *m*CPBA (715 mg, 2.90 mmol, 70% pure) in dichloromethane (10 mL) was dried on $MgSO_4$ and added to a solution of **3b** (1.03 g, 2.90 mmol) in dichloromethane (10 mL), while kept at 0^0C. The reaction mixture was stirred for 1h, washed with satd. $NaHCO_3$ (2x), water (1x) and brine (1x), dried ($MgSO_4$) and concentrated *in vacuo*. The residual oil was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:3) to give sulfoxide **4b** (1.02 g, 95%) as a colorless oil. R_f 0.23 (hexane/ethyl acetate 1:3); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.38-2.81 (m, 2H, $SCH_2C\underline{H}_2$), 2.84-3.10 (m, 2H, $SC\underline{H}_2CH_2$), 3.70 (s, 3H, OC$\underline{H}_3$), 4.88-5.03 (m, 1H, C$\underline{H}$N), 7.31-7.53 (m, 5H, SPh), 7.57-7.78 (m, 4H, phthaloyl) ppm.

Pummerer reaction of ***4a,b*** *using acetic anhydride (general procedure)*
A solution of **4a** or **4b** (2.0 mmol) in acetic anhydride (40 mL) in the absence of a catalyst (entry 1, Table 1) or in the presence of *p*-toluenesulfonic acid hydrate (0.01 eq or 0.1 eq; entries 2, 5-6, Table 1) was stirred under the indicated conditions (Table 1). Work-up was accomplished by removing the solvent *in vacuo*, and dissolving the residue in a mixture of ethyl acetate and satd. $NaHCO_3$. The organic phase was washed with satd. $NaHCO_3$ (2x), water (1x) and brine (1x), dried ($MgSO_4$), and concentrated *in vacuo*. The product distribution was determined by ^{1}H-NMR analysis.

Methyl 4-acetoxy-2-(phthaloylimino)-4-methylsulfanyl-butanoate (**5a**) and *methyl 4-acetoxymethylsulfanyl-2-(phthaloylimino)-butanoate* (**7a**)
These compounds were obtained as an inseparable mixture in quantitative yield (entry 1, Table 1) **5a** (1:1 mixture of diastereomers): R_f 0.23 (hexane/ethyl acetate 2:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.99, 2.10, 2.16, 2.18 (4 s, 6H, O(O)CC$\underline{H}_3$ and SC$\underline{H}_3$), 2.51-2.88 (m, 2H, C$\underline{H}_2$), 3.74 (s, 3H, OC$\underline{H}_3$), 5.07 (m, 1H, C$\underline{H}$N), 5.74-5.91 (m, 1H, SC$\underline{H}$O), 7.72-7.94 (m, 4H, phthaloyl) ppm.
7a: R_f 0.23 (hexane/ethyl acetate 2:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.06 (s, 3H, O(O)CC$\underline{H}_3$), 2.44-2.88 (m, 4H, C$\underline{H}_2$), 3.74 (s, 3H, OC$\underline{H}_3$), 5.07 (m, 1H, C$\underline{H}$N), 5.13 (s, 2H, OC$\underline{H}_2$S), 7.72-7.94 (m, 4H, phthaloyl) ppm.

Methyl 2-(phthaloylimino)-4-methylsulfanyl-but-3-enoate (**6a**) and (**7a**) (entry 2, Table 1).
Purification by flash chromatography (SiO_2, hexane/ethyl acetate 2:1) provided **7a** and **6a** (mixture of *E*- and *Z*-isomers) as colorless oils in a yield of 77%.
E-**6a** and *Z*-**6a**: R_f 0.35 (hexane/ethyl acetate 2:1); ^{1}H-NMR data were in complete agreement with those reported previously.[13]

Methyl 4-acetoxy-2-(phthaloylimino)-4-phenylsulfanyl-butanoate (**5c**) and *methyl 2-(phthaloylimino)-4-phenylsulfanyl-but-3-enoate* (**6b**) (entries 5 and 6, Table 1)
These compounds were separated by flash chromatography (SiO_2, hexane/ethyl acetate 3:1) to provide **5c** (1:1 mixture of diastereomers) and **6b** (mixture of *E*- and *Z*-isomers) as colorless oils in 73-80% yield.
5c (1:1 mixture of diastereomers): R_f 0.17 (hexane/ethyl acetate 3:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.96, 2.06 (2xs, 3H, O(O)CC$\underline{H}_3$), 2.51-3.03 (m, 2H, C$\underline{H}_2$), 3.71 (s, 3H, OC$\underline{H}_3$), 5.00, 5.09 (2 dd, 1H, J 5.3 Hz, J 10.5 Hz, C$\underline{H}$N), 5.94, 5.98 (2 dd, 1H, J 4.6 Hz, J 9.8 Hz, SC$\underline{H}$O), 7.27-7.57 (m, 5H, SPh), 7.67-7.92 (m, 4H, phthaloyl) ppm.
E-**6b** and *Z*-**6b**: R_f 0.23 (hexane/ethyl acetate 3:1); ^{1}H-NMR data were in complete agreement with those reported previously.[13]

Pummerer reaction of ***4a,b*** *using trifluoroacetic anhydride (general procedure)*
To a solution of **4a** or **4b** (0.25 mmol) in dichloromethane (5 mL) TFAA (1.5 eq.) was added. The solution was stirred for 30 min at room temperature and concentrated *in vacuo*. The residue was dissolved in dichloromethane and again concentrated *in vacuo*. The product distribution of the crude reaction mixture, obtained as a colorless oil, was determined by ^{1}H-NMR analysis.

Methyl 2-(phthaloylimino)-4-trifluoroacetoxymethylsulfanyl-butanoate (**7b**) and (**6a**) (entry 3, Table 1)
7b: ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.72 (m, 4H, C$\underline{H}_2$), 3.75 (s, 3H, OC$\underline{H}_3$), 5.06 (m, 1H, C$\underline{H}$N), 5.43 (s, 2H, OC$\underline{H}_2$S), 7.84 (m, 4H, phthaloyl) ppm.
E-**6a** and *Z*-**6a**: R_f 0.35 (hexane/ethyl acetate 2:1); ^{1}H-NMR data were in complete agreement with those reported previously.[13]

Methyl 2-(phthaloylimino)-4-phenylsulfanyl-4-trifluoroacetoxy-butanoate (**5d**) and (**6b**) (entry 7, Table 1)
5d (mixture of two diastereomers): ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 2.80 (m, 2H, C$\underline{H}_2$), 3.72 (s, 3H, OC$\underline{H}_3$), 5.13 (m, 1H, C$\underline{H}$N), 6.14 (m, 1H, SC$\underline{H}$O), 7.33 (m, 5H, SPh), 7.79 (m, 4H, phthaloyl) ppm.
E-**6b** and *Z*-**6b**: R_f 0.23 (hexane/ethyl acetate 3:1); ^{1}H-NMR data were in complete agreement with those reported previously.[13]

1-[N-(tert-butyloxycarbonyl)-1-amino-2-hydroxyethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**13b**)
A solution of **13a**[18] (4.53 g, 11.0 mmol) was dissolved in a mixture of dichloromethane (45 mL), methanol (5 mL), piperidine (5mL) and stirred for 18h at room temperature. The volatiles were removed *in vacuo* and the residue was dissolved in dichloromethane (50 mL), followed by the addition of Boc_2O (3.83 g, 17.5 mmol) and DMAP (134 mg, 1.10 mmol). After 18h of stirring at room temperature, the solution was washed with satd. NH_4Cl (2x) and satd. $NaHCO_3$ (2x). Drying ($MgSO_4$) and careful concentration *in vacuo* afforded an oil, which was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:2). Yield 1.21 g (38%) of **13b** as a colorless oil. R_f 0.35 (hexane/ethyl acetate 1:2); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 0.83 (s, 3H, C$\underline{H}_3$), 1.45 (s, 9H, C$\underline{H}_3$ *tert*-butyl), 2.65 (br s, 1H, O$\underline{H}$), 3.63-3.93 (m, 3H, C$\underline{H}$N + C$\underline{H}_2$OH), 3.93 (s, 6H, C$\underline{H}_2$), 5.08 (br d, 1H, J 7.9 Hz, N$\underline{H}$) ppm; IR (CCl_4): ν 3560 (OH), 3450 (NH), 1710 (C=O) cm^{-1}; MS (CI,

m/z, rel. int. (%)): 290 ([M+1]$^+$, 8.1), 234 ([$C_9H_{16}NO_6$]$^+$, 74.0), 216 ([$C_9H_{14}NO_5$]$^+$, 100), 158 ([$C_7H_{12}NO_3$]$^+$, 67.4), 57 ([C_4H_9]$^+$, 52.6).

1-[N-(tert-butyloxycarbonyl)-1-amino-2-oxoethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**11b**)

To a stirred solution of oxalyl chloride (790 mg, 6.22 mmol) in dichloromethane (50 mL) was added DMSO (875 mg, 11.2 mmol) at -78^{0}C under nitrogen. Then a solution of **13b** (900 mg, 3.11 mmol) in dichloromethane (15 mL) was gradually added. Stirring was continued for 2h at -78^{0}C, followed by the addition of di*iso*propyl ethylamine (3.30 mL, 18.9 mmol). After 1h stirring at 0^{0}C, dichloromethane (200 mL) was added and the solution was washed with ice-cold satd. NH_4Cl (2x) and brine (1x), dried ($MgSO_4$), and carefully concentrated *in vacuo* to give **11b** as a colorless oil in quantitative yield, which was sufficiently pure for further reactions. Purity according to capillary GC 96%; R_f 0.55 (hexane/ethyl acetate 1:2); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 0.83 (s, 3H, C$\underline{H}_3$), 1.44 (s, 9H, C$\underline{H}_3$ *tert*-butyl), 3.95 (s, 6H, C$\underline{H}_2$), 4.50 (d, 1H, J 8.5 Hz, C$\underline{H}$N), 5.11 (br d, 1H, J 8.5 Hz, N$\underline{H}$), 9.67 (s, 1H, C$\underline{H}$O) ppm; IR (CCl_4): ν 3450 (NH), 1720 (C=O) cm^{-1}; MS (CI, m/z, rel. int. (%)): 288 ([M+1]$^+$, 2.7), 232 ([$C_9H_{14}NO_6$]$^+$, 100), 158 ([$C_7H_{12}NO_3$]$^+$, 73.8), 57 ([C_4H_9]$^+$, 84.0).

Peterson olefination of 11b

To a cooled (0^{0}C) solution of silane **17**[23] (988 mg, 5.03 mmol) in THF (12 mL) was gradually added a solution of *n*-butyllithium (5.1 mmol, 1.6M in hexane) in a nitrogen atmosphere. After 1h stirring at 0^{0}C, a solution of aldehyde **11b** (682 mg, 2.37 mmol) in THF was added dropwise and stirring was continued for 2h at room temperature. The solution was quenched with satd. NH_4Cl and the aqueous phase was extracted with diethyl ether (3x). The combined organic extracts were washed with brine (1x), dried ($MgSO_4$) and carefully concentrated *in vacuo*. The residual oil was purified by flash chromatography (SiO_2, hexane/ethyl acetate 3:1) to give *Z*-**14** (202 mg, 22%) and *E*-**14** (261 mg, 28%) as white solids.

Z-**14**: R_f 0.25 (hexane/ethyl acetate 2:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 0.81 (s, 3H, C$\underline{H}_3$), 1.45 (s, 9H, C$\underline{H}_3$ *tert*-butyl), 3.93 (s, 6H, C$\underline{H}_2$), 4.66-4.96 (m, 2H, C$\underline{H}$N and N$\underline{H}$), 5.69 (dd, 1H, J 8.0 Hz, J 9.6 Hz, SCH=C$\underline{H}$), 6.43 (d, 1H, J 9.6 Hz, SC$\underline{H}$=CH), 7.20-7.41 (m, 5H, SPh) ppm; IR (CCl_4): ν 3450 (NH), 1720 (C=O) cm^{-1}; MS (EI, m/z, rel. int. (%)): 393 ([M]$^+$, 36.4), 337 ([$C_{16}H_{19}NO_5S$]$^+$, 100), 184 ([$C_9H_{14}NO_3$]$^+$, 20.6), 110 ([C_6H_6S]$^+$, 23.4), 57 ([C_4H_9]$^+$, 56.8).

E-**14**: R_f 0.27 (hexane/ethyl acetate 2:1); ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 0.81 (s, 3H, C$\underline{H}_3$), 1.45 (s, 9H, C$\underline{H}_3$ *tert*-butyl), 3.91 (s, 6H, C$\underline{H}_2$), 4.41 (m, 1H, C$\underline{H}$N), 4.92 (br d, 1H, J 8.8 Hz, N$\underline{H}$), 5.93 (dd, 1H, J 5.9 Hz, J 15.2 Hz, SCH=C$\underline{H}$), 6.40 (dd, 1H, J 15.2 Hz, ^{4}J 1.0 Hz, SC$\underline{H}$=CH), 7.21-7.34 (m, 5H, SPh) ppm; IR (CCl_4): ν 3455 (NH), 1715 (C=O) cm^{-1}; Mass data were the same as for *Z*-**14**.

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Chapter 14*

Involvement of Ethene in the Induction of Germination by GR24 in *Striga hermonthica*

Abstract: The aim of this chapter is to gain insight in the mechanism of action of the strigol analog GR24 on the stimulation of germination and ethene production of *Striga hermonthica* seeds Ethene production was measured by a photoacoustic method in a flowing air system, which allows ultra-sensitive monitoring of ethene under physiological conditions Ethene production and germination correlate very well when stimulated by a racemic diastereomeric mixture of GR24, and by the enantiopure stereoisomers (+)-GR24 and (-)-GR24 Exogenous 1-aminocyclopropane-1-carboxylic acid (ACC), which is the precursor of ethene, gave ethene production in a dose dependent way However, germination percentages were much lower in comparison with those induced by GR24 concentrations producing similar amounts of ethene GR24-induced ethene production was hardly affected by the presence of exogenous ACC The observation that only high (unphysiological) concentrations of ACC and ethene trigger germination disfavors the hypothesis that GR24 exerts its stimulatory effect by inducing the biosynthesis of ethene

14.1 Introduction

Background

Root parasite flowering plants of the genera *Striga* (Scophulariaceae) and *Orobanche* (Orobanchaceae) have developed an interesting chemical ecology, as they require various host-derived recognition cues, such as exposure to a germination stimulant [1] Following the isolation and identification of (+)-strigol from the roots of the false host cotton (*Gossypium hirsutum* L) as a highly active naturally occurring germination stimulant,[2] several very potent synthetic analogs have been prepared, *e g* GR24 [3,4] Despite the insight in the structural requirements of strigol analogs to retain germination stimulatory activity,[5] hardly anything is known about their mode of action

Ethene has been reported to stimulate germination of seeds of various *Striga* species,[6-8] but not of *Orobanche* [9] These observations have led to the hypothesis that strigol and its analogs,

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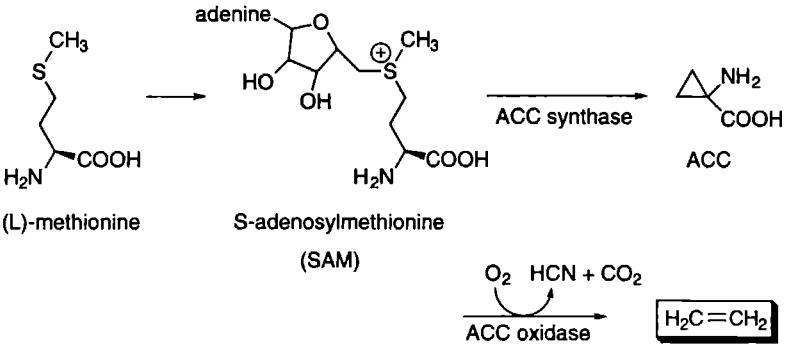
including GR24, trigger germination by eliciting the biosynthesis of ethene in *Striga*.[10-13] A better understanding of the biochemical processes involved in the germination of seeds of *Striga* and *Orobanche* may be of value for the design of novel stimulants and/or inhibitors as well as the development of new parasite control strategies.

Biosynthesis of ethene

The generation of ethene in higher plants occurs almost exclusively via conversion of (L)-methionine into 1-aminocyclopropane-1-carboxylic acid (ACC) and subsequent oxidation as depicted in scheme 1.[14]

Scheme 1



The formation of ACC from SAM (scheme 1) is mediated by the pyridoxal enzyme ACC synthase, which is strongly inhibited by β,γ-unsaturated amino acids, such as aminoethoxyvinyl glycine (AVG), a known inhibitor of pyridoxal phosphate-mediated enzyme reactions.[15] The mechanism of inhibition is discussed in chapter 13. The conversion of SAM into ACC is generally regarded as the rate-determining step in the biosynthesis of ethene.[16] The final step is the oxidation of ACC. This step requires O_2 and is inhibited by Co(II) ions or an anaerobic atmosphere. It may be assumed that ethene binds to some component of the cell in order to exert its physiological effects. Although an ethene receptor has never been characterized, it is known that certain inhibitors of ethene action, such as 2,5-norbornadiene (NBE) displace ethene from ethene binding proteins.[16]

This chapter describes preliminary experiments to evaluate the current hypothesis that strigol analogs induce germination by eliciting the biosynthesis of ethene. In addition, experiments are described to gain insight in the role of strigol analogs in the biochemical cascade leading to ethene production. The release of ethene from the seeds is monitored by a photoacoustic technique in a flowing air system, as well as by the usual gas chromatographic

method.[17] The former technique offers the unique opportunity to measure the kinetics of ethene production under physiological conditions in an ultra-sensitive way.

14.2 Materials and Methods

Seed treatments and chemicals

Seeds of *S. hermonthica* (Del.) Benth. were received from Dr. A.G.T. Babiker and had been collected in Sudan in 1987. Seed surface sterilization and conditioning were performed as previously described.[18] GR24 as a 1:1 mixture of diastereomers, *i.e.* (±)-GR24, was prepared as reported previously.[4] A fresh 100 ppm stock solution was prepared by dissolving 10.0 mg GR24 in 10 mL of acetone p.a. and dilution with demineralized water to 100 mL. Appropriate dilutions were made as is indicated.

The preparation of (+)-GR24 ($[\alpha]_D$=+436^0, in chloroform) and (-)-GR24 ($[\alpha]_D$=-446^0, in chloroform) has been described in chapter 5. 1-Aminocyclopropane-1-carboxylic acid (ACC) was prepared according to an in house developed procedure (unpublished results).

Ethene measurements by GC

"Double discs" of glass fibre filter paper (1 cm diameter), each with approximately 80 conditioned (14 days at 29^{0}C) *S. hermonthica* seeds were placed in 6 mL vials (10 double discs per vial). Each double disc was wetted with 100 μΛ of test solution. Vials were sealed with rubber septa and placed in an incubator at 29^{0}C for 24 h unless mentioned otherwise. At the end of the incubation period a sample of headspace gas (0.5 mL) was drawn from each vial. Ethene was determined by gas chromatography using an Hayesep N packed column and a flame ionization detector. The corresponding germination percentages were assessed immediately after ethene determinations in the same vials by counting all germinated seeds. There were two duplicates per treatment and all experiments were repeated at least twice. In all experiments seeds were treated both with 0.1% acetone and with 0.01 ppm GR24 as controls.

Data presented are from single experiments unless indicated otherwise. Interpretation of data was complicated by variable responses upon treatment of the seeds with GR24 during the time of the year.[18]

Photoacoustic measurements of ethene

Ethene production was measured by a photoacoustic method in a flowing air system, the set-up of which is depicted in fig 1. The laser photoacoustic trace detection of gases in the atmosphere is a sensitive and reliable method for molecular gases which absorb in the infrared.[19-21] By using the photoacoustic set-up a three orders of magnitude higher sensitivity compared to gaschromatographic methods is obtained. Therefore, accumulation and pretreatment effects can be avoided by using a flowing air system through the sampling volume.[21-24]

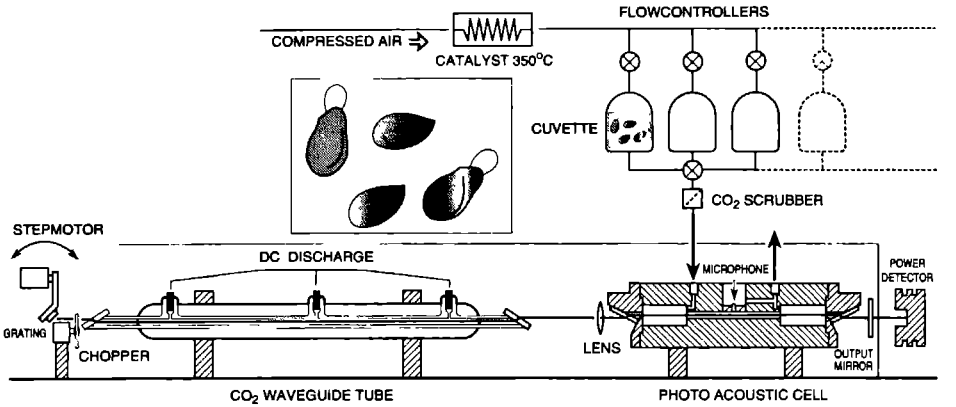


Figure 1. Laser driven photoacoustic set-up

Approximately 2400 seeds were placed in vials (6 mL), which were incubated at 29^{0}C Before air (flow-rate = 0.8 l/h) entered the sampling cell it was passed through a catalyst in order to remove saturated hydrocarbons. Between the sampling cell and the photoacoustic cell were placed a KOH based scrubber in order to remove CO_2 from the air and an additional cooling trap (-150^{0}C), which eliminates ethanol. As radiation source an infrared CO_2 waveguide laser was used. Due to the strong absorption of ethene at the laser wavelengths it is possible to detect very low concentrations in the air.

The photoacoustic effect (*i.e.* the transformation of light energy into acoustic energy) is based on the fact that molecules absorb electromagnetic radiation. As a consequence they are excited to a higher energy level. De-excitation occurs via non-radiative decay, which increases the kinetic energy and temperature of the gas molecules. When this process takes place in a constant volume the pressure is increased. When the light source is chopped with an audiofrequency, pressure fluctuations of the same frequency occur, which are detectable with a microphone.

14.3 Results

In order to determine the activity-range of GR24 , dose-response curves were determined of both germination and the stimulation of ethene production, using seeds of *Striga hermonthica*. In the first experiment both (±)-GR24 (fig 2) (a mixture of all four stereoisomers), and (+)-GR24 possessing the same stereochemistry as naturally occurring (+)-strigol, were included (fig 3). The most striking observation is the very similar response of both germination and ethene production within the concentration range tested, which can be expressed in terms of a corresponding ED50 value (dose at half-maximal effect) for both parameters. This means that, in principle, ethene production is a good indicator to assess the germination stimulatory activity toward *Striga hermonthica* seeds induced by strigol analogs. Optically pure (+)-GR24 is only slightly more active than (±)-GR24, which is the result of the lower concentration of the more active

stereoisomer in the mixture of isomers. Another observation is the complete lack of activity upon exposure to high concentrations of GR24, which is reflected in both germination and ethene production This phenomenon of supraoptimal stimulant concentration has often been encountered in the literature [25] This inactivity, most likely, results from toxic side-effects, although direct evidence is lacking

The activity of the optical antipode (-)-GR24 was next examined In order to make relevant comparisons , (+)-GR24 was included in the same experiment (fig 4) The presented data reveal that (-)-GR24 has to be approximately 100 times more concentrated to be as active as the "natural" isomer Again, germination and ethene production correlate very well, which is the case for both isomers Aqueous controls showed low activity in both experiments

In order to monitor time-dependent release of ethene, seeds were incubated in a flowing air system as is described in Materials and Methods Ethene production rates obtained from seeds which had been treated with an aqueous control (0 1% acetone) did not differ from an empty cuvet The difference curve obtained from a GR24-treated sample (0 1 ppm) and an empty cuvet is shown in fig 5 After a lag time of 6h major ethene production started and ceased completely after 19h This main peak was preceded by a minor increase of ethene production with a maximum after 3h, which was found to be reproducible During the experiment germination was assessed No germination was visible during the first eight hours of the experiment, whereas the germination percentage was already maximal after 17h

In order to establish the effect of exogenous ACC, seeds were incubated with different concentrations of ACC (fig 6) It was found that high concentrations of ACC are able to enhance ethene production and to stimulate germination, although the response to GR24 was rather small in these experiments (poor quality of seeds)

In order to establish the effect of exogenous ACC in combination with GR24, seeds were incubated with ACC in the presence and absence of GR24 at the indicated concentrations (fig 7) GR24 (0 01 ppm) and ACC (0 1 mM) show comparable ethene producing activities However, germination percentages of GR24 stimulated seeds were much higher Incubation with both compounds resulted in increased ethene production and a slightly higher germination percentage when compared to the GR24 treatment alone A similar experiment was conducted using ACC (0 1 mM) and a concentration range of GR24 (table 1) When using a sub-optimal concentration of GR24 (10^{-3} mg/L), its activity is considerably increased by the presence of ACC

In order to follow the kinetics of ethene production in more detail, a similar experiment was conducted, employing the photoacoustic set-up (fig 8) Again a lag-time of 6h preceded initial ethene evolution, which was independent of the treatment Addition of both GR24 and ACC gave a substantial increase of the initial and maximal rate of ethene production Ethene production starts at the same time in all cases and is thus independent of the treatment Moreover, the ethene response elicited by ACC and GR24, applied together, seems to be a cumulation of the effects of the separate treatments This result also follows from the data depicted in fig 7 Whereas ethene

production in the presence of only GR24 ceased completely after 20h (fig 8), it continued for an additional period of at least 24h when ACC was included (data not shown).

Table 1. Effect of GR24 and ACC on *Striga hermonthica* seed germination and ethene production [a]

treatment	germination (%)	ethene production[b]
GR24 (2.10^{-2} mg/L)	57.6 ± 1.1	1.06
GR24 (2.10^{-3} mg/L)	36.7 ± 1 1	0.86
GR24 (2.10^{-4} mg/L)	8.3 ± 1.2	0.25
ACC (0.1 mM)	27.8 ± 5.0	3.17
GR24 (10^{-2} mg/L) + ACC (0 1 mM)	57 3 ± 1 9	5.84
GR24 (10^{-3} mg/L) + ACC (0.1 mM)	50.7 ± 1.4	4 45
GR24 (10^{-4} mg/L) + ACC (0 1 mM)	31.2 ± 2.6	3.09
aqueous control	4.2 ± 0.9	0 11

a) 10 "Double discs" were placed in 6 mL vials After addition of the stimulant(s), the vials were incubated and assayed for ethene production (GC detection) and germination (see materials and methods) Data presented are from one single experiment ± S E

b) Amount of ethene, expressed in nanoliters, produced by *ca* 450 seeds placed in 6 mL vials after 24 h of incubation

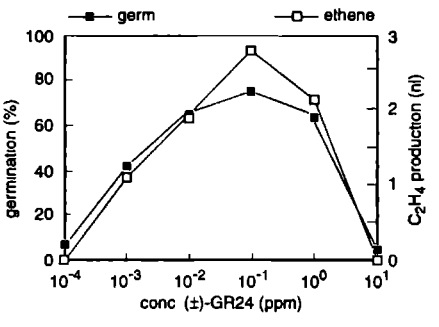


Figure 2. Effect of the concentration of (±)-GR24 on *S hermonthica* seed germination and ethene production Headspace gas was sampled 24h after incubation and assayed for ethene by GC Germination was examined immediately after each ethene determination

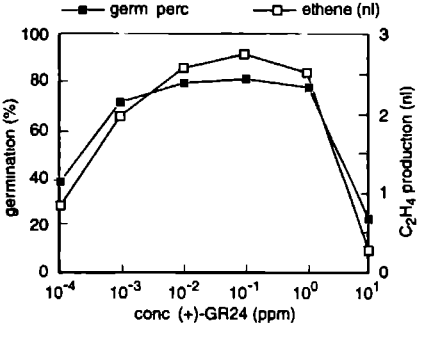


Figure 3. Effect of the concentration of (+)-GR24 on *S hermonthica* seed germination and ethene production Headspace gas was sampled 24h after incubation and assayed for ethene by GC Germination was examined immediately after each ethene determination

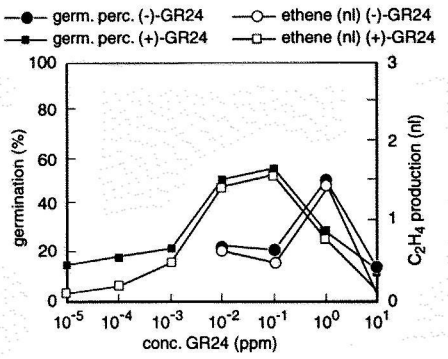


Figure 4. Effects of (+)-GR24 and (-)-GR24 concentrations on *S. hermonthica* seed germination and ethene production. Headspace gas was sampled 24h after incubation and assayed for ethene by GC. Germination was examined immediately after each ethene determination.

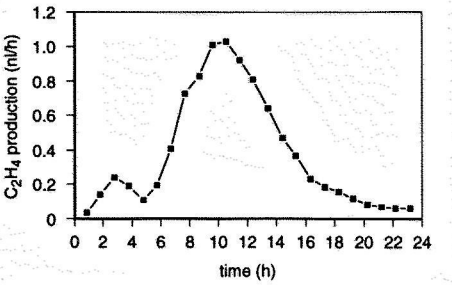


Figure 5. Kinetics of ethene production by *S. hermonthica* seeds stimulated by GR24 (0.1 ppm). Immediately after GR24 treatment seeds were incubated and assayed for the rate of ethene production using the photoacoustic set-up as is explained in Materials and Methods.

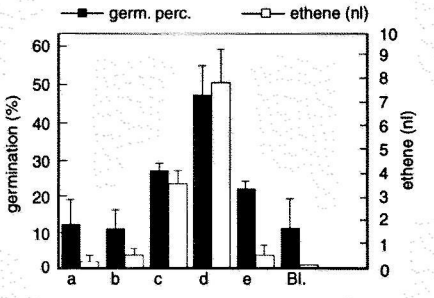


Figure 6. Effects of ACC concentrations and GR24 (0.01 ppm) concentrations on *S. hermonthica* seed germination and ethene production. Headspace gas was sampled 24h after incubation and assayed for ethene by GC. Germination was examined immediately after each ethene determination. Data presented are means ± SE of four separate experiments. Each sample was replicated in duplicate.
a, ACC (0.01 mM); b, ACC (0.1 mM); c, ACC (1 mM); d, ACC (10 mM); e, GR24 (0.01 ppm); Bl.: aqueous control

14.4 Discussion

For the present discussion some recent relevant literature reports should be taken into account. Parker and Jackson[11] proposed a function for the action of ethene in the GR24-induced seed germination of *Striga hermonthica*, whereas *Striga forbesii* did not show such a requirement. This conclusion was based on the observation that 2,5-norbornadiene (NBE), an inhibitor of ethene action, suppressed the germination of *Striga hermonthica* seeds, while the production of ethene was only little affected. This means that ethene generation is <u>not</u> simply a consequence of germination stimulated by the strigol analog. By including various inhibitors (AVG, $CoCl_2$, NBE) interfering at different stages of the ethene cascade, Logan and Stewart[10] suggested that sorghum root exudate stimulates germination by promoting the conversion of SAM into ACC, *i.e.* induction of ACC-synthase. In contrast, based on similar experiments Babiker *et al.*[12] argued that strigol triggers the germination of *Striga asiatica* by activating ACC-oxidase.

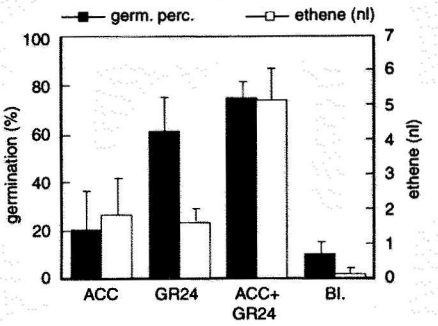


Figure 7. Effects of GR24 (0.01 ppm), ACC (0.1 mM) and GR24 (0.01 ppm) + ACC (0.1 mM) on *S. hermonthica* seed germination and ethene production. Headspace gas was sampled 24h after incubation and assayed for ethene by GC. Germination was examined immediately after each ethene determination. Data presented are means ± SE of four separate experiments. Each sample was replicated in duplicate.

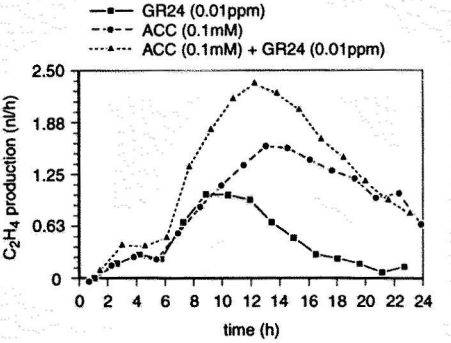


Figure 8. Kinetics of ethene production by *S. hermonthica* seeds stimulated by GR24 (0.01 ppm), ACC (0.1 mM) and GR24 (0.01 ppm) + ACC (0.1 mM). Immediately after treatment seeds were incubated and assayed for the rate of ethene production using the photoacoustic set-up as is explained in Materials and Methods.

The results presented here show that the stimulation of germination of seeds of *Striga hermonthica* by (+)-GR24, (-)-GR24 and a mixture of the four possible stereoisomers, is accompanied by ethene generation, which can be expressed in quantitative terms (figs. 2-4). The time course of ethene production corresponds rather well with seed germination and is independent of the (active) GR24 concentration, as was demonstrated by some photo-acoustic measurements using different concentrations ($1\text{-}10^{-3}$ ppm) of GR24 (data not shown). Major ethene release and visible germination are preceded by a small initial production level (fig 5). It is tempting to speculate that this small amount of ethene acts as a trigger, and autocatalytically enhances main ethene production. Autocatalytic effects of ethene are rather common in nature.[16] The data presented in figs. 5-8 and table 1 reveal that ACC at concentrations of 0.1 mM and higher, when supplied alone, is able to elicit ethene production and to stimulate germination. This observation leads to the suggestion that ACC or its precursors indeed is the limiting factor to trigger germination. In other words, according to Logan and Stewart, ACC-synthase is induced by GR24 to give ACC. This is subsequently oxidized to ethene, which is the ultimate trigger leading to germination.[10] However, it should be noted that very high (unphysiological) concentrations of ACC and ethene are required to mimic the effect of GR24. The amount of ACC added is a factor 10^4 higher than the amount of ethene produced after the addition of GR24; and the amount of ethene released under 'natural' conditions is at least a factor 100 lower than that needed to mimic the effect of GR24. Concentrations of ACC and GR24, which generate similar levels of ethene (*cf.* fig. 7), give unrelated germination percentages; the latter compound is considerably more active. Moreover, the very similar kinetics of ethene production (fig. 8) during

the first 8 hours after addition of GR24 or ACC disfavors Logan and Stewart's hypothesis that a strigol analog induces *de novo* synthesis of ACC-synthase.[10] It should be noted that no direct experimental evidence has been provided by these authors. Also the possibility of a direct effect of GR24 on ACC-oxidase as proposed by Babiker *et al.*[12,13] is unlikely, because the effects exerted by ACC and GR24 are virtually independent of each other (figs. 7, 8; table 1). The present study thus strongly disfavors the hypothesis that strigol analogs exert their biological activity at the level of the biosynthesis of ethene.

On the other hand, there is abundant evidence[10,11] that ethene is capable of triggering the germination of seeds of *Striga hermonthica*. However, it should be emphasized that the concentration of ethene produced through the action of GR24 (*vide supra*) is simply far too low to trigger germination (at least a factor 100). A conceivable explanation for a possible role of ethene in the germination process is that the sensitivity to ethene is increased by strigol analogs, *i.e.* ethene binding proteins are activated through a synergistic effect. Very low levels of ethene then can stimulate germination and induce feed-back mechanisms to generate the amounts of ethene, which are actually measured. This mechanism is, however, independent of the interaction of GR24 with a receptor protein.

The requirement for endogenous ethene is apparently not general for all parasitic weeds. Similar experiments using seeds of *Orobanche crenata* did not result in the production of detectable amounts ethene upon the addition of GR24 (1 mg/L). Moreover, ACC (0.1 mM) did not stimulate germination, nor did AVG (0.1 mM) inhibit GR24-induced germination (data not shown). Therefore, compounds that are precursors or inducers of ethene are potential germination stimulants of seeds of *Striga hermonthica*, but not for *Orobanche crenata* seeds.

In conclusion, there is an effect of ethene on the seed germination of *Striga hermonthica*. Whether this role is connected with that of strigol and its analogs remains uncertain. Ethene, however, cannot be the sole trigger of the germination by strigol and its analogs. This study convincingly shows that there is no direct effect of GR24 in the biosynthesis of ethene. Another reason why an effect of GR24 in the biosynthesis of ethene is unlikely is that not all seeds of parasitic weed species produce ethene or are responsive to ethene (*e.g. Orobanche crenata* or *Striga forbesii*), whereas the seeds of these species are stimulated by the same germinating agents that induce germination of parasitic species that are responsive to ethene.

14.5 References

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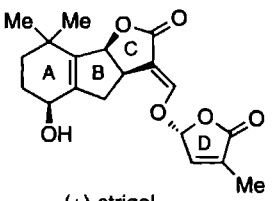
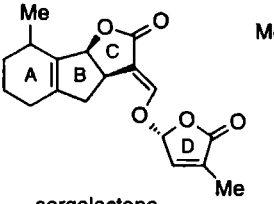
Summary

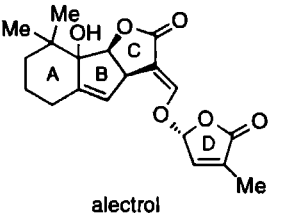
This thesis deals with the stimulating agents for the germination of seeds of the parasitic angiosperms *Striga* and *Orobanche* spp. These weeds parasitize mainly monocotyledonous food crops (*e.g.* mais, sorghum, millet, etc.) and dicotyledonous plants (*e.g.* tomatoes, faba beans, sun flower, etc.), respectively. The seeds of these parasitic weeds only germinate when they are exposed to a germination stimulant, which is exuded by the roots of a potential host plant. Thus far, three naturally occurring germination stimulants belonging to the 'strigolactone' family are known, *viz.* (+)-strigol, sorgolactone and alectrol.



(+)-strigol



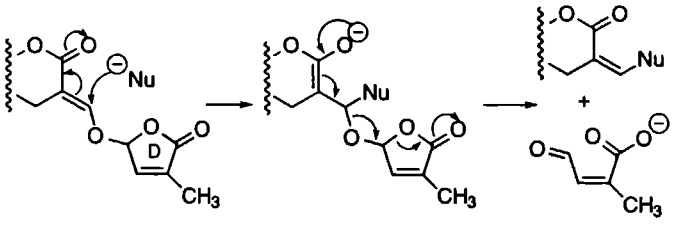
sorgolactone



alectrol

These compounds have three structural units in common, namely the C-ring, the connecting vinyl ether moiety and the D-ring. During previous research extensive attention has been given to the identification of the bioactiphore in these strigolactones, *i.e.* that part of these molecules that is primarily responsible for the biological activity. A molecular mechanism for the initial stages of the germination has been proposed which involves an addition/elimination reaction induced by a nucleophilic site in the receptor, as is depicted in scheme 1.

Scheme 1

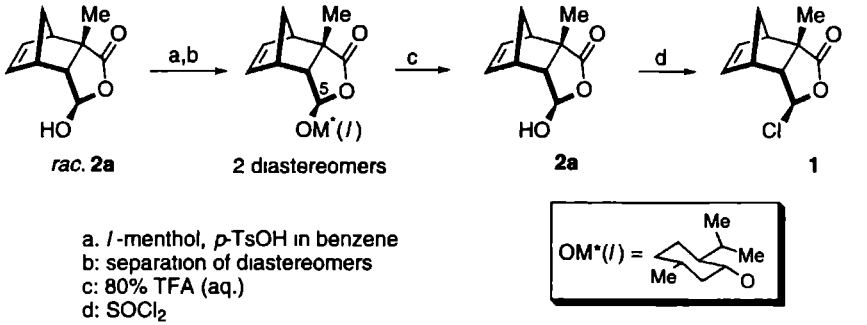


In **chapter 1** a brief general introduction is presented, which is based on several recent reviews on this subject. The life-cycle of the parasitic weeds, the interaction with the host plant, control strategies of weed pests and structure-activity relationships of strigol and its analogs are described.

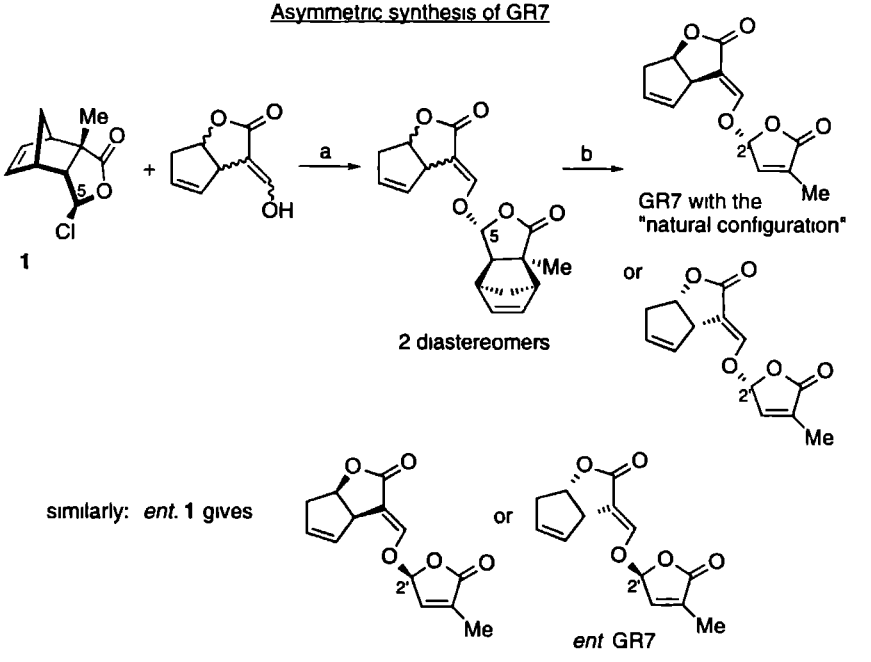
In the **chapters 2-6** the focus is on the stereochemistry of strigol analogs, especially on their stereocontrolled synthesis.

Chapter 2 is devoted to the synthesis of all four stereoisomers of the strigol analog GR7, involving the strategic use of a homochiral latent D-ring precursor, *viz. endo*-tricyclic-*exo*-chloro lactone **1**. The synthesis of this latent D-ring fragment involves the resolution of *exo*-hydroxy lactone *rac.* **2** using *l*-menthol as the chiral auxiliary (scheme 2). The antipode of **1**, *i.e. ent.* **1**, was similarly prepared using *d*-menthol in the resolution process.

Scheme 2



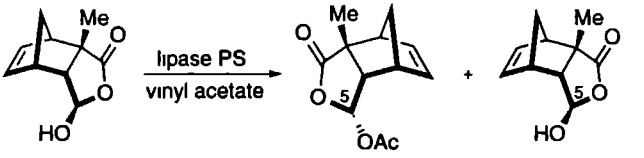
Scheme 3



Enantiopure **1** and *ent* **1** were utilized in the synthesis of all four stereoisomers of GR7, as is outlined in scheme 3. Coupling of the racemic BC-fragment with either enantiopure **1** or *ent* **1**, followed by chromatographic separation of the diastereomers thus obtained and subsequent thermal cycloreversion in *o*-dichlorobenzene at 180^{0}C gave the enantiopure stereoisomers

In **chapter 3** a considerably improved resolution of hydroxy lactone *rac* **2a** is described, whereby effective use is made of a lipase PS catalyzed kinetic resolution in the presence of vinyl acetate as the irreversible acetyl donor (scheme 4) A remarkable epimerization at C-5 took place during this enzymatic acetylation.

Scheme 4



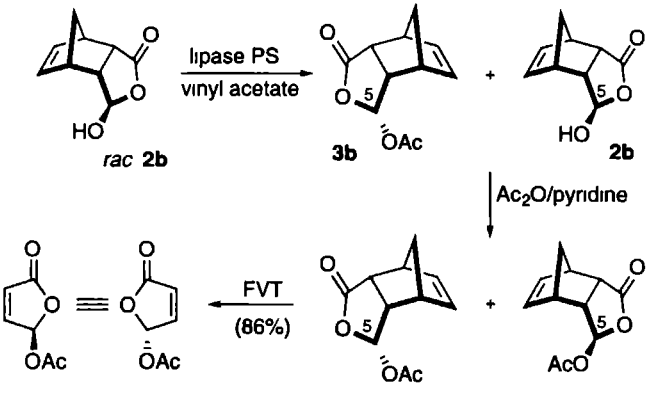
rac **2a** **3a** **2a**

e e 87% e e 85%

(56% conversion)

This unexpected reaction can be explained by invoking an equilibrium beween the tricyclic *exo*- and *endo*-hydroxy lactone (mutarotation) and a much faster enzymatic acetylation of the *endo*-isomer The exclusive formation of the *endo*-acetate **3a** is a nice example of the Curtin-Hammett principle This enzymatic process could elegantly be elaborated to a chiral economical synthesis of 5-acetoxy-furanone **4a**, as is outlined in scheme 5.

Scheme 5

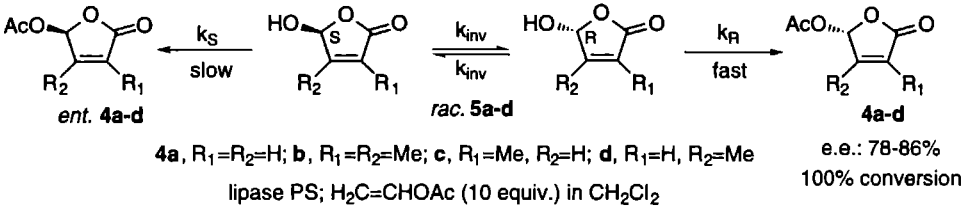


rac **2b** **3b** **2b**

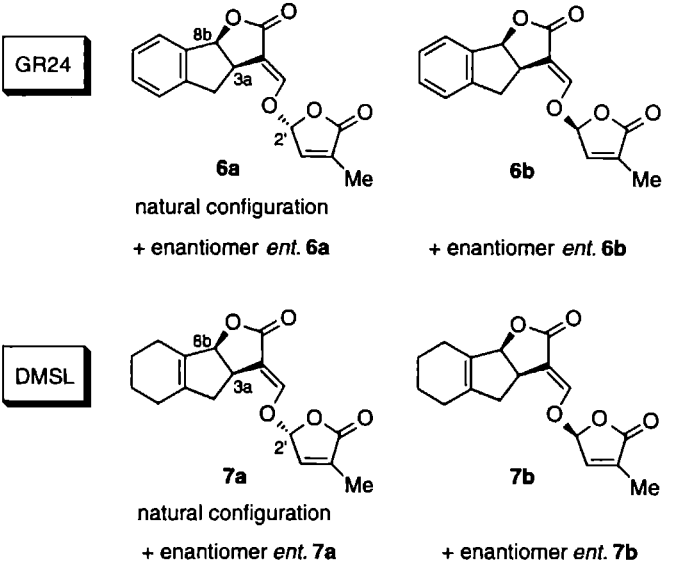
4a, e e =94% c.y 95% (from *rac* **2b**)

Another efficient synthesis of 5-acetoxy-furanones **4** in high enantiopurity is presented in **chapter 4**. By means of a dynamic kinetic resolution of 5-hydroxy-2(5H)-furanones *rac.* **5a-d** using lipase PS in the presence of vinyl acetate, acetoxy-furanones **4a-d** were obtained in a chiral economical manner (scheme 6).

Scheme 6

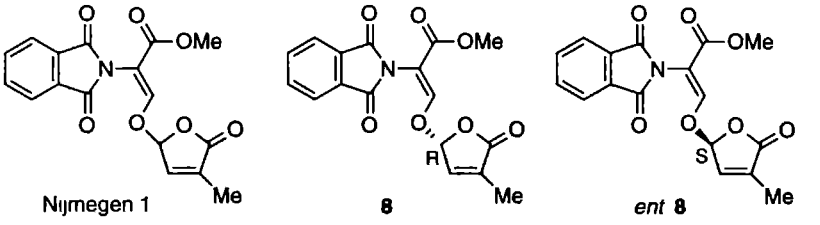


Chapters 5 and **6** are devoted to the asymmetric synthesis of all four stereoisomers of the strigol analogs GR24 (**6**) and desmethyl sorgolactone (DMSL, **7**), respectively, successfully applying the synthetic strategy that was introduced in chapter 2. From the bioassays of the respective stereoisomers it was concluded that the isomer with the natural configuration of (+)-strigol at both stereogenic centers (C-3a,C-8b and C-2') exhibits the highest germination activity toward seeds of *Striga hermonthica* and *Orobanche crenata*. The optical antipodes of these 'natural' isomers showed a stimulatry activity which is at least a factor of 100 lower.



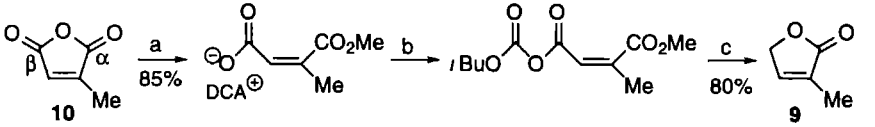
In **chapter 7** the synthesis of a new strigol analog, namely Nijmegen 1, is reported. The enantioselective synthesis of both enatiomers of this ABD-analog was accomplished again

making use of the strategy which is described in chapter 2 This relatively simple analog **8** has a considerable stimulatory activity in the germination of seeds of *Striga hermonthica* and *Orobanche crenata* Again the enantiomer with the natural configuration at C-2' is most active The enantiomers **8** and *ent* **8** show significant differences in germination activity



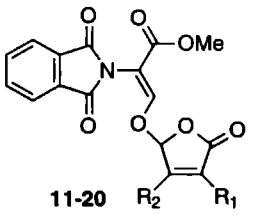
In **chapter 8** a new synthesis of the important D-ring precursor 3-methyl-2(5H)-furanone (**9**) is described Citraconic anhydride, which is a cheap starting material, is treated with dicyclohexylamine and then converted into a mixed anhydride which is subsequently subjected to reduction and ring closure (scheme 7)

Schema 7

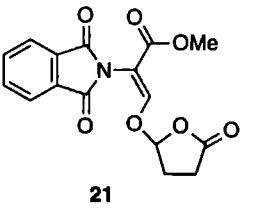


a MeOH, dicyclohexylamine (DCA)
b Et_3N, $ClCO_2tBu$
c $NaBH_4$ in THF/H_2O

The structural modifications of D-ring are in focus in **chapter 9**, thereby employing the basic skeleton of Nijmegen 1 The new D-ring analogs **11-22** were prepared and tested on their ability to induce germination of seeds of *Striga hermonthica* and *Orobanche crenata* It was found that structural variations in the D-ring have an enormous influence on the germinating activity Only the dimethyl substituted analog **15** and the ethyl substituted compound **17** have a bioactivity toward germination of seeds of *Striga hermonthica* comparable with that of Nijmegen 1 However, the germination activity of **15** and **17** toward *Orobanche* seeds is strongly diminished in comparison with Nijmegen 1 The D-ring analog **15** is an attractive candidate for the control of weed pests, as its preparation is relatively simple and its activity is appreciable For this purpose the concept of suicidal germination may be appopriate, *i e* treatment of the soil with stimulant in the absence of the host plant, the germinated seeds will be unable to develop further due to the lack of a host



11-20 R_2 R_1



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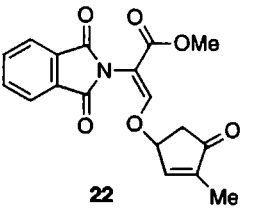
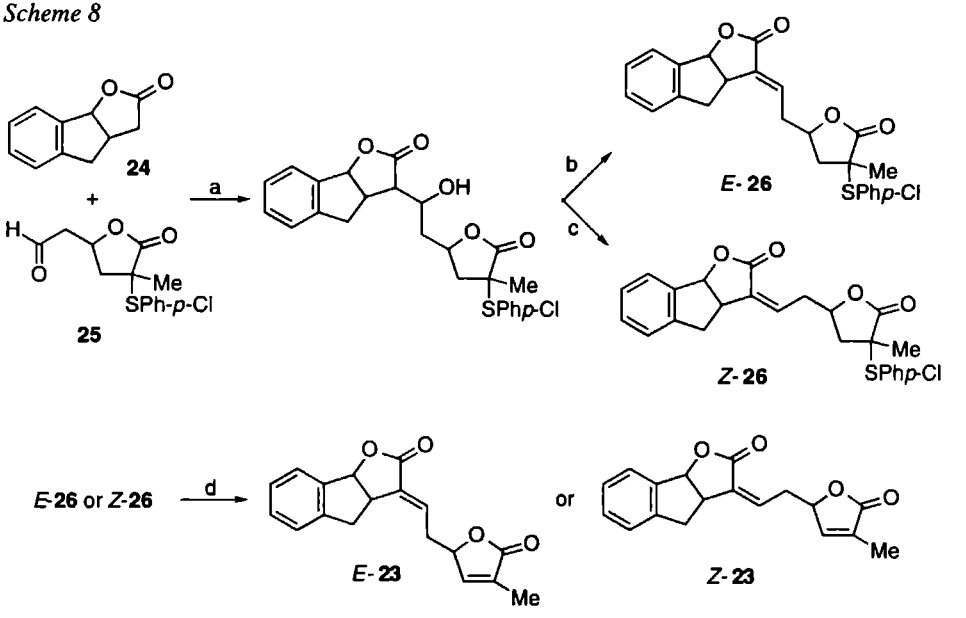


22

11 R_1=Me, R_2=H
12 - R_1- R_2 - = - - CH=CH-CH=CH-
13 R_1=H, R_2=H
14 R_1=H, R_2=Me
15 R_1=Me, R_2=Me
16 R_1=Me, R_2=OMe
17 R_1=Et, R_2=H
18 R_1=Ph, R_2=H
19 R_1=H, R_2=Ph
20 R_1=Cl, R_2=Cl

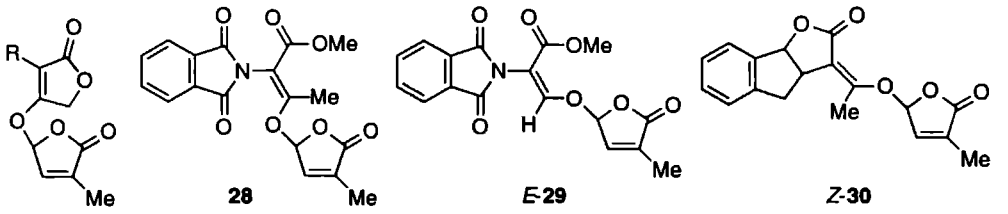
The **chapters 10** and **11** deal with structural variations in the vinyl ether part of the strigol analogs The synthesis of *E*- and *Z*-carba GR24 (*E*-**23** and *Z*-**23**), which is described in **chapter 10**, proceeds by a condensation of the ABC-fragment **24** with the unstable aldehyde **25** The olefinic bond was introduced by an elimination reaction, in the case of *Z*-**26** via a mesylate and for *E*-**26** via a reaction with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (scheme 8) Both isomers of carba-GR24 are entirely inactive in inducing germination of seeds of parasitic weeds This result is a firm support for the molecular mechanism shown in scheme 1.

Scheme 8



In **chapter 11** various strigol analogs with modifications in the vinyl enol ether moiety are described The new analogs include tetronic acid derived analogs **27**, which have an endocyclic

enol ether double bond, methyl substituted Nijmegen 1 derivative **28**, *E*-Nijmegen 1 (*E*-**29**), and methyl substituted *Z*-GR24 (*Z*-**30**).

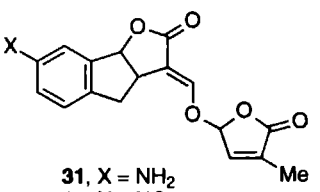


27a R=H
27b R=Me
27c R=Ph

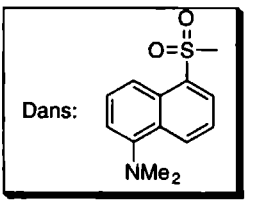
28 *E*-**29** *Z*-**30**

Bioassays revealed that the analogs **27-29** exhibit an appreciable activity in the stimulation of the germination of *Striga hermonthica* and *Orobanche crenata* seeds, implying that the substitution pattern of the enol ether moiety is not very critical for the bioactivity.

In **chapter 12** the synthesis of GR24 analogs are described which contain a label or a photoreactive group in the A-ring. These strigol analogs can, at least in principle, be used for the purpose of identifying the receptor protein. The key compound in the synthesis of this type of analogs is amino-GR24 (**31**), which is accessible by the mild nitration of the ABC-lactone of GR24. This amino compound could readily be converted into the dansyl derivative **33**, which represents a GR24-analog containing a fluorescent label. It is of utmost importance that this labeled compound **33** exhibits a high germination activity on seeds of *Striga hermonthica* (not of *Orobanche crenata*). Apparently the presence of a large substituent in the A-ring of GR24 does not influence its bioactivity. Therefore, this compound **33** may serve its purpose in the protein fishing experiments.



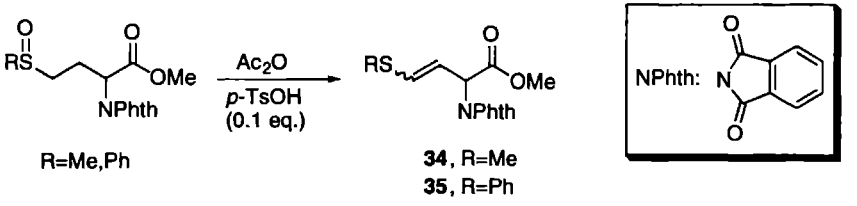
31, X = NH_2
32, X = NO_2
33, X = -NHDans



In **chapter 13** two approaches are described for the synthesis of sulfur analogs of aminoethoxyvinyl glycine (AVG). These compounds are of interest in connection with the inhibition of the ethene production during the germination process. These AVG analogs may be instrumental in gaining insight in the role of ethene during germination and possibly in the control of parasitic weed pests. By means of a Pummerer reaction of an appropriate methionine

derivative an AVG analog was obtained, still containing its protecting groups (scheme 9). Removal of these protecting groups from **34** and **35** has not been investigated as yet.

Scheme 9

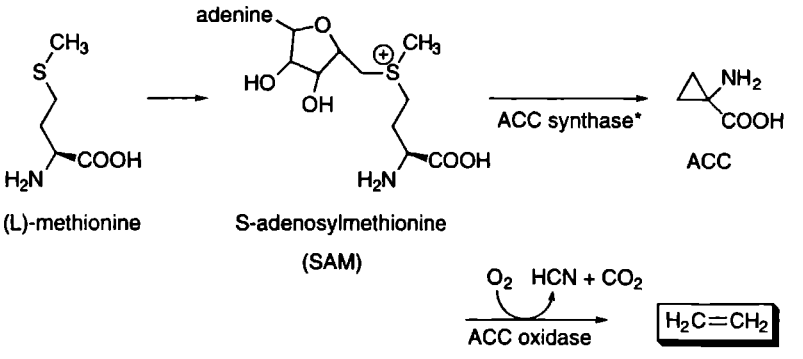


R=Me,Ph

34, R=Me
35, R=Ph

In the final **chapter** (**14**) explicit attention is given to the role of ethene during the induction of germination of seeds of parasitic weeds. Ethene is produced from methionine via the intermediacy of 1-amino-1-carboxylic acid (ACC) as is shown in scheme 10. The production of ethene during the germination process cannot be attributed to a direct effect of the germination stimulant GR24 in one or more of the biosynthetic steps of ethene, as has been suggested previously. Ethene is not the prime trigger of the germination induced by strigol and its analogs. It may be hypothesized, however, that ethene has an effect in the further biochemical cascade leading to germination of seeds of *Striga hermonthica* spp.

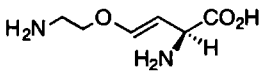
Scheme 10



(L)-methionine

S-adenosylmethionine (SAM)

* This step can be inhibited by AVG



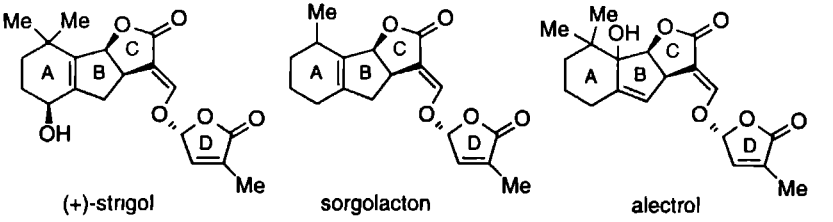
AVG

Concluding remarks

The research described in this thesis has gained new insights into the structure-activity relationship of analogs of strigol regarding the stimulation of germination of seeds of *Striga* and *Orobanche*. A general synthetic strategy has been developed, from which all possible stereoisomers of strigol analogs can be obtained in enantiomerically pure form. From biological tests it was proved that the absolute stereochemistry in the ABC-part has great influence upon the activity. Modifications in the D-ring (substitutions and stereochemistry) were also found to have a large effect. These results suggest that the D-ring as well as the AB-fragment are bound in the receptor. This complexation is essential for germination stimulatory activity. Modification of the α,β-unsaturated vinyl ether moiety led to the conclusion that the intrinsic ability of these compounds to undergo an addition-elimination reaction (scheme 1) is crucial for stimulatory activity. A synthetic methodology has been developed which enables the introduction of a wide variety of labels in the A-ring with the retention of activity. This is a significant step toward the identification of a receptor protein for strigol. Attention has been particularly focussed on the biochemical working mechanism of strigol analogs. The effect of these analogs turns out to be independent of and not coupled to the production of ethene, as has been previously suggested.

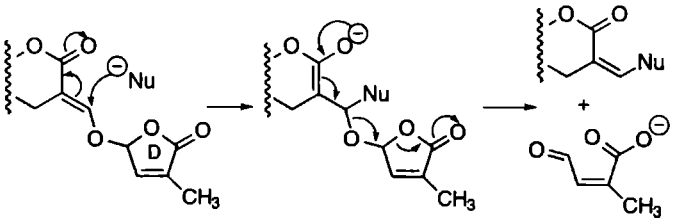
Samenvatting

Dit proefschrift handelt over de stimulantia voor de ontkieming van zaden van de parasitaire naaktzadigen *Striga* en *Orobanche* spp Deze onkruiden parasiteren voornamelijk éénzaadlobbige voedselgewassen (mais, sorghum, gierst, etc), respectievelijk tweezaadlobbige planten, zoals tomaten, tuinbonen, zonnebloemen, etc. De zaden van deze parasitaire planten kunnen alleen ontkiemen als ze in contact komen met een kiemstimulant, die gewoonlijk wordt afgescheiden door de wortels van een potentiele waardplant Tot nu toe zijn er drie natuurlijk voorkomende kiemstimulanten bekend, die allen behoren tot de familie van de "strigolactonen", namelijk (+)-strigol, sorgolacton en alectrol



Deze verbindingen hebben drie gemeenschappelijke structuureenheden, namelijk de C-ring, de verbindende vinyl ether groep en de D-ring In eerder onderzoek werd reeds uitgebreid aandacht besteed aan de identificatie van de bioactifoor in deze strigolactonen, d w z het gedeelte van deze moleculen dat primair verantwoordelijk is voor de biologische activiteit Dit heeft geleid tot het voorstel voor een moleculair mechanisme van de ontkieming, dat inhoudt dat een nucleofiel centrum in de receptor een additie-eliminatie reactie in gang zet, zoals is weergegeven in schema 1

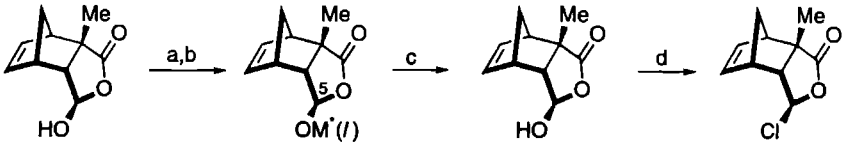
Schema 1



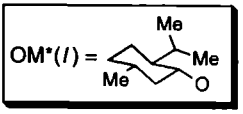
In **hoofdstuk 1** wordt een korte algemene inleiding gegeven op basis van recent verschenen overzichtsartikelen Hierbij komen de levenscyclus van het parasitaire onkruid en de interactie met de gastheerplant aan de orde, alsmede bestrijdingsmethoden en structuur-activiteits relaties van strigol en analoga

In de **hoofdstukken 2-6** wordt ingegaan op de stereochemie van de strigol-analoga, met name op de synthese er van. **Hoofdstuk 2** is gewijd aan de bereiding van alle vier stereoisomeren van het analogon GR7, waarbij gebruik wordt gemaakt van een homochirale latente D-ring precursor, te weten *endo*-tricyclo-*exo*-chloorlacton **1**. Voor de synthese van dit latente D-ringfragment wordt een resolutie van *exo*-hydroxylacton *rac.* **2a** bewerkstelligd met behulp van *l*-menthol als chirale hulpverbinding (schema 2)

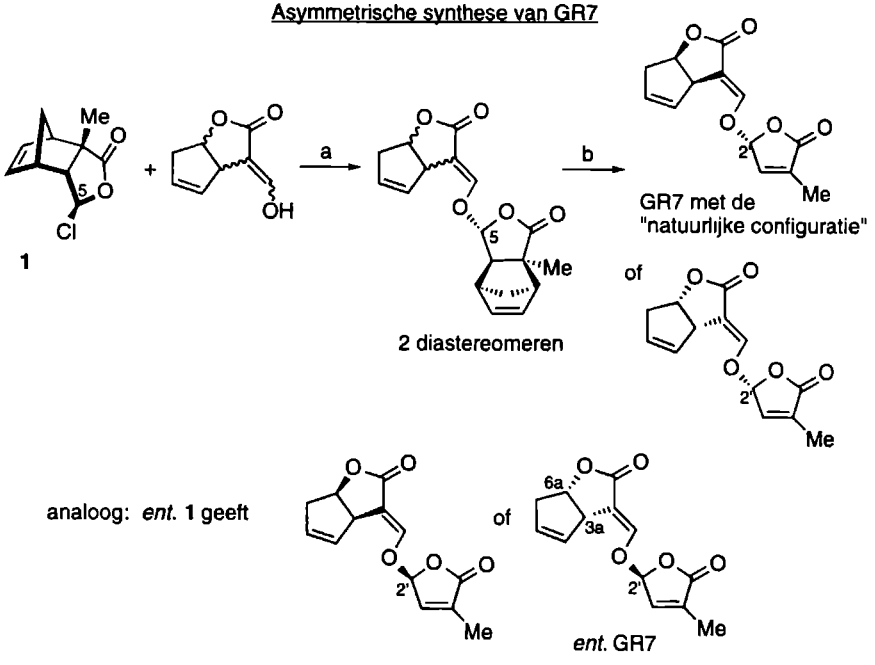
Schema 2



a: *l*-menthol, *p*-TsOH in benzeen
b: scheiding van diastereomeren
c: 80% TFA (aq.)
d: $SOCl_2$



Schema 3

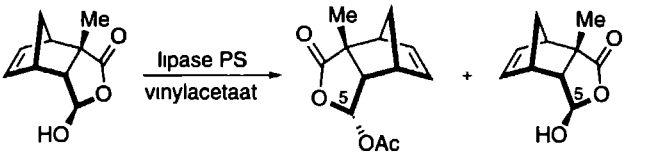


a: KO^{t}Bu, DMF, 20h, scheiding van diastereoisomeren
b: *o*-dichloorbenzeen, 180^{0}C.

De antipode van **1**, dit is *ent* **1**, werd bereid gebruikmakend van *d*-menthol De enantiomeer-zuivere D-ring precursors **1** en *ent* **1** werden benut bij de synthese van de vier stereoisomeren van GR7, zoals is weergegeven in schema 3 Na de koppeling met het racemische BC-fragment met **1** respectievelijk *ent* **1** en scheiding van de gevormde diastereomeren, volgde een thermische cycloreversie in *o*-dichloorbenzeen als laatste stap

In **hoofdstuk 3** wordt een aanzienlijke verbeterde resolutie van *rac* **2a** beschreven, gebruikmakend van een lipase PS gekatalyseerde kinetische resolutie in aanwezigheid van vinyl acetaat als irreversibele acetyldonor (schema 4)

Schema 4



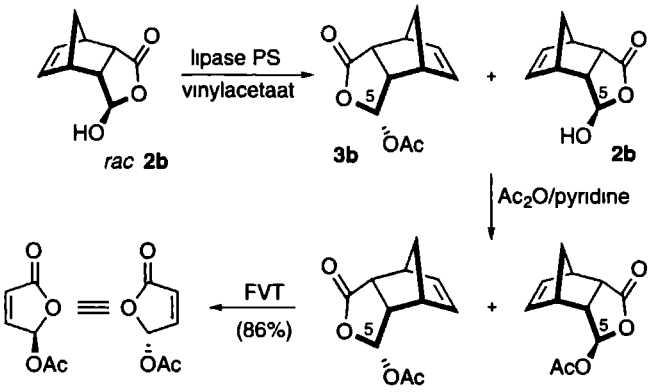
rac **2a** **3a** **2a**

e e 87% e e 85%

(56% conversie)

Hierbij bleek een verrassende epimerisatie op C-5 plaats te vinden Deze bijzondere reactie kan verklaard worden door een evenwicht van *exo*- en *endo* tricyclisch lacton aan te nemen (mutarotatie), waarbij de *endo*-vorm zeer veel sneller reageert in de enzymatische acetylering Deze exclusieve vorming van *endo*-acetaat **3a** is een fraai voorbeeld van het Curtin-Hammett principe Dit enzymatische proces kon op elegante wijze worden benut voor een chiraal-economische synthese van 5-acetoxy-2(5H)-furanon (**4a**), zoals is weergegeven in schema 5

Schema 5

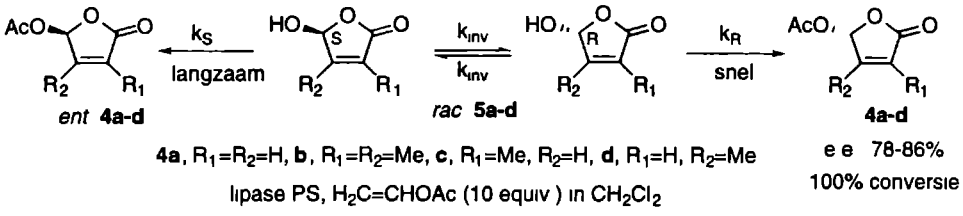


rac **2b** **3b** **2b**

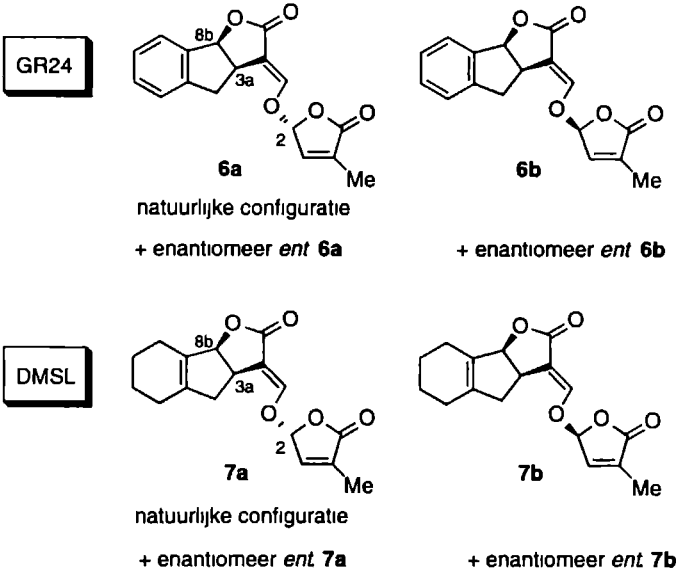
4a, e e =94% opbrengst 95% (uit *rac* **2b**)

Een andere efficiente synthese van 5-acetoxy-2(5H)-furanon **4a** met hoge enantiomere zuiverheid staat beschreven in **hoofdstuk 4** Door middel van een dynamische kinetische resolutie van 5-hydroxy-2(5H)-furanonen **5a-d** met behulp van lipase PS in aanwezigheid van vinylacetaat werden de acetoxy-furanonen **4a-d** op een chiraal economische wijze verkregen (schema 6)

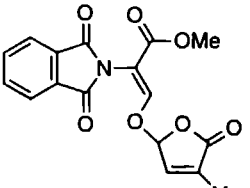
Schema 6



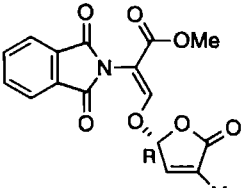
De **hoofdstukken 5** en **6** zijn gewijd aan de asymmetrische synthese van alle vier stereoisomeren van de strigol-analoga GR24 (**6**) respectievelijk desmethyl sorgolacton (DMSL, **7**), waarbij de synthese-strategie die werd geintroduceerd in hoofdstuk 2 wederom met succes werd toegepast Uit bioassays van deze stereoisomeren werd geconcludeerd dat het isomeer met de natuurlijke configuratie van (+)-strigol op de beide stereogene centra (C-3a,C-8b en C-2') de hoogste kiemingsactiviteit heeft voor zaden van *Striga hermonthica* en *Orobanche crenata* De optische antipoden van deze "natuurlijke" isomeren vertonen een kiemingsactiviteit die ten minste een factor 100 lager is



In **hoofdstuk 7** wordt de synthese van een nieuw strigol-analogon, namelijk Nijmegen 1, beschreven De enantioselectieve synthese van beide antipoden van dit ABD-analogon werd gerealiseerd gebruikmakend van de strategie die in hoofdstuk 2 werd ontwikkeld. Dit relatief eenvoudige analogon heeft een aanzienlijke biologische activiteit bij de kieming van zaden van *Striga hermonthica* en *Orobanche crenata*, waarbij het enantiomeer met de "natuurlijke" configuratie op C-2' (**8**) de beste kiemresultaten geeft



Nijmegen 1



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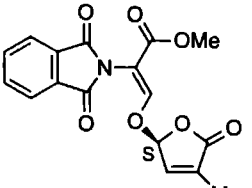
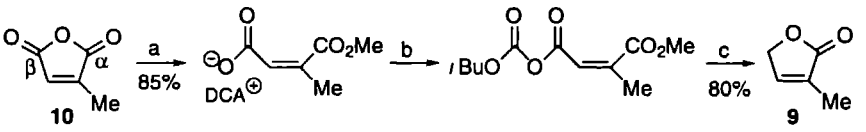


ent **8**

In **hoofdstuk 8** wordt een nieuwe synthese van de D-ringprecursor 3-methyl-2(5H)-furanon (**9**) gepresenteerd De goedkope uitgangsstof citraconzuuranhydride (**10**) wordt eerst behandeld met dicyclohexylamine, dan omgezet in een gemengd anhydride en vervolgens gereduceerd en weer ringgesloten (schema 7).

Schema 7

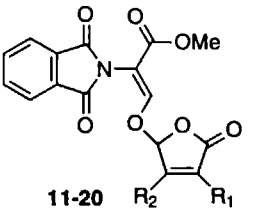


10 **9**

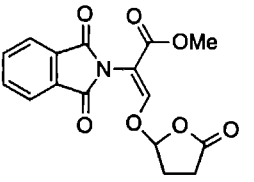
a MeOH, dicyclohexylamine (DCA)
b Et_3N, $ClCO_2tBu$
c $NaBH_4$ in THF/H_2O

De structurele modificaties van de D-ring vormen het onderwerp van **hoofdstuk 9**, waarbij het basisskelet van Nijmegen 1 werd gebruikt. De D-ring analoga **11-22** zijn allen getest op hun vermogen kieming van *Striga hermonthica* en *Orobanche crenata* zaden te bewerkstelligen. Het bleek dat structurele variaties in de D-ring een grote invloed hebben op de kiemingsactiviteit Alleen het dimethylgesubstitueerde analogon **15** en de ethylgesubstitueerd verbinding **17** bleken een zelfde activiteit als Nijmegen 1 te hebben bij de kieming van *Striga hermonthica*, echter voor *Orobanche crenata* bleek deze structurele modificaties te leiden tot sterk verminderde activiteit in vergelijking met Nijmegen 1. Het D-ring-analogon **15** is op grond van de goede kiemstimulering en de eenvoudige synthese een aantrekkelijke kandidaat voor het gebruik bij de bestrijding van parasitaire onkruidplagen volgens het concept van de suicidale kieming, d w z behandeling van

de teeltgrond met stimulant in afwezigheid van het gewas, waardoor de ontkiemende zaden zich niet kunnen ontwikkelen bij gebrek aan een gastheer



11-20



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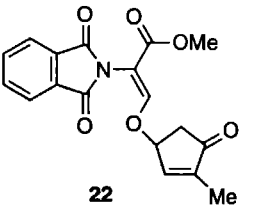


22

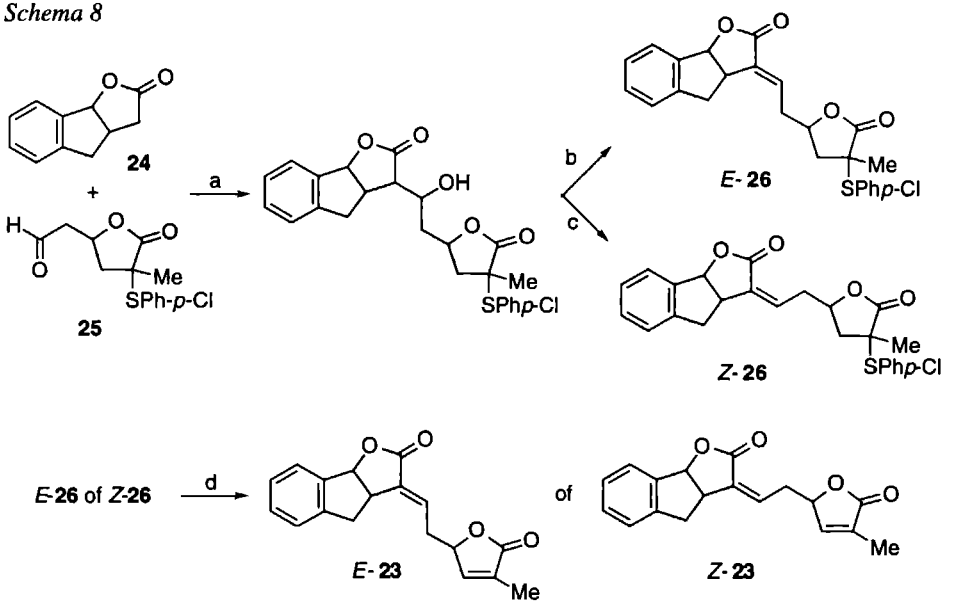
11 R_1=Me, R_2=H
12 - R_1- R_2 - = - - CH=CH-CH=CH-
13 R_1=H, R_2=H
14 R_1=H, R_2=Me

15 R_1=Me, R_2=Me
16 R_1=Me, R_2=OMe
17 R_1=Et, R_2=H

18 R_1=Ph, R_2=H
19 R_1=H, R_2=Ph
20 R_1=Cl, R_2=Cl

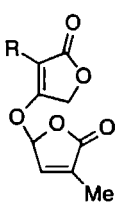
De **hoofdstukken 10** en **11** zijn gewijd aan structurele modificaties in het vinylethergedeelte van strigol analoga De synthese van *E*- en *Z*-carba GR24 (*E*-**23** en *Z*-**23**), zoals vermeld in **hoofdstuk 10**, verloopt via een condensatie van het ABC-fragment **24** met het instabiele aldehyde **25** De olefinische band werd geintroduceerd m b v een eliminatiereactie, *Z*-**26** werd bereid via het mesylaat en *E*-**26** via reactie met 2-fluor-1-methylpyridinium *p*-tolueensulfonaat (schema 8) Beide isomeren *E*-**23** en *Z*-**23** bleken geheel inactief te zijn bij de kieming van *Striga hermonthica* en *Orobanche crenata* zaden Dit resultaat is een sterke ondersteuning van het moleculair mechanisme dat in schema 1 is weergegeven

Schema 8

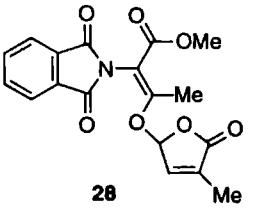


a LDA in THF
b 2-fluoro-1-methylpyridinium *p*-toluenesulfonaat
c 1 MsCl Et_3N, 2 DBU
d 1 *m*CPBA, 2 Δ

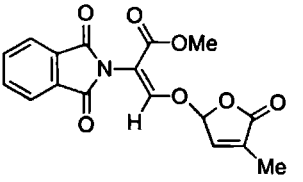
In **hoofdstuk 11** worden strigol-analoga beschreven met diverse modificaties in het vinylether-gedeelte Bereid werden de tetronzuurderivaten **27a-c**, die een endocyclische enolether dubbele band bezitten, methyl gesubstitueerd Nijmegen 1 derivaat **28**, *E*-Nijmegen 1 (*E*-**29**), en methyl gesubstitueerd Z-GR24 (Z-**30**)



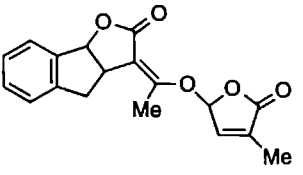
27a R=H
27b R=Me
27c R=Ph



28



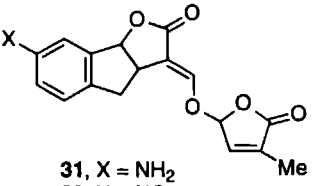
E-**29**



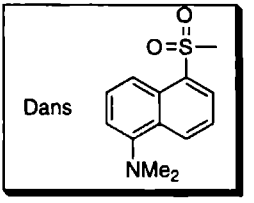
Z-**30**

Uit de biologische evaluatie van de analoga **27-29** bleek dat het substitutiepatroon van de enoletherband niet erg kritisch is voor de biologische activiteit

In **hoofdstuk 12** wordt de synthese van GR24 analoga beschreven die een label of een fotoreactieve groep bezitten Deze strigol-analoga kunnen in principe gebruikt worden voor de identificatie van een strigolreceptor eiwit De sleutelverbinding in deze synthese is het amino-GR24 (**31**), dat gemaakt kan worden via een nitrering van het ABC-lacton van GR24 Deze amino verbinding kan worden omgezet in het dansylderivaat **33**, waardoor een GR24-analogon met een fluorescerend label is verkregen Van buitengewoon groot belang is dat deze dansylverbinding een zeer hoge biologische activiteit vertoont bij de kiemimg van *Striga hermonthica* zaden Kennelijk wordt de biologische activiteit van GR24 niet sterk beinvloed door door een dergelijke substitutie van de A-ring Derhalve is dit analogon bruikbaar in "protein fishing" experimenten



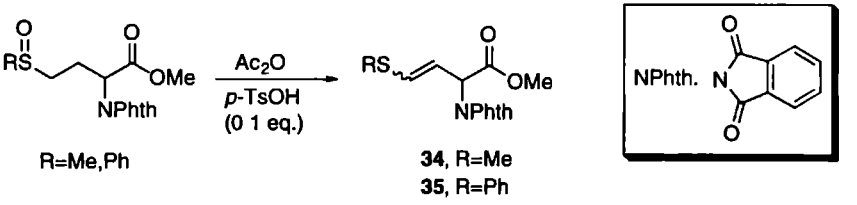
31, X = NH_2
32, X = NO_2
33, X = -NHDans



In **hoofdstuk 13** worden twee benaderingen beschreven voor de synthese van zwavelanaloga van aminoethoxyvinyl-glycine (AVG) Deze verbindingen zijn van interesse i v m de remming van de etheenproductie die plaats vindt tijdens het kiemingsproces Deze AVG-analoga zijn van belang om meer inzicht te verwerven in de rol van etheen tijdens de kieming en eventueel van de bestrijding van parasitaire onkruiden Door een Pummerer reactie

van een geschikt methionine-derivaat werd een beschermd AVG-analogon verkregen (schema 9) De ontscherming van **34** en **35** is tot AVG-analoga werd nog niet onderzocht.

Schema 9

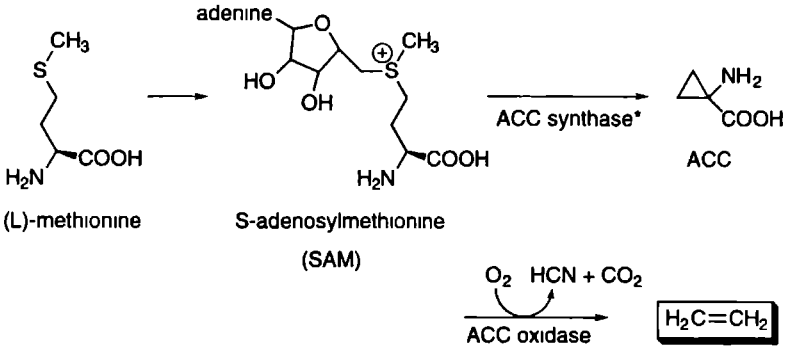


R=Me,Ph

34, R=Me
35, R=Ph

In het laatste **hoofdstuk (14)** wordt ingegaan op de rol van etheen bij de inductie van de kieming van parasitaire onkruidzaden. Etheen wordt geproduceerd uit methionine via aminocyclopropaancarbonzuur (ACC) als intermediair (schema 10). De productie van etheen tijdens het kiemingsproces kan niet worden toegeschreven aan een direct effect van GR24 op één van de stappen in de biosynthese ervan, zoals recent werd gesuggereerd Verder is etheen zeker niet de primaire trigger bij de stimulering van kieming door strigol-analoga. Het is wel aannemelijk dat etheen een rol speelt in de verdere biochemische cascade die leidt tot ontkieming van *Striga hermonthica* zaden

Schema 10

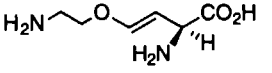


(L)-methionine

S-adenosylmethionine (SAM)

ACC

* Deze stap kan worden geremd door AVG



AVG

Slotopmerking

Het onderzoek beschreven in dit proefschrift heeft geleid tot een aantal nieuwe inzichten in de structuur-activiteitsrelatie van strigol-analoga bij de kiemingsstimulering van zaden van *Striga* en *Orobanche*. Er is een algemeen toepasbare synthese-strategie ontwikkeld, waarmee alle mogelijke stereoisomeren van strigol-analoga in enantiomeer-zuivere vorm verkregen kunnen worden. Uit biologische testen kon geconcludeerd worden dat de absolute stereochemie in het ABC-gedeelte van grote invloed is op de activiteit. De marge in de modificatie van de D-ring (substituties en stereochemie) is daarentegen zeer gering. Deze resultaten leiden tot de suggestie dat zowel de D-ring als het AB-fragment een affiniteit met een receptor hebben, die essentieel is voor de kiemingsactiviteit. Uit de bestudering van modificaties in het α,β-onverzadigde vinylether gedeelte bleek dat het intrinsieke vermogen van deze verbindingen om een additie-eliminatie reactie (schema 1) te ondergaan cruciaal is voor de kiemingsactiviteit. Er is een synthese-methodologie ontwikkeld waarmee diverse labels in de A-ring geintroduceerd kunnen worden met behoud van de kiemingsactiviteit. Dit is een belangrijke stap op weg naar de identificatie van een receptoreiwit voor strigol. Tenslotte is er aandacht besteed aan het biochemische werkingsmechanisme van strigol-analoga. Het effect van deze analoga blijkt op zichzelf te staan en niet gekoppeld te zijn aan de productie van etheen, zoals eerder werd gesuggereerd.

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Curriculum Vitae

Jan Willem Thuring werd geboren op 10 mei 1966 te Jos (Nigeria). Van 1978 tot 1984 bezocht hij het Titus Brandsmalyceum te Oss, waar hij het gymnasium-β diploma behaalde. Na het propaedeutisch jaar van de Hogere Laboratorium School te Oss in 1985 te hebben afgerond, werd begonnen met de studie scheikunde aan de Katholieke Universiteit Nijmegen. In juli 1986 werd het propaedeutisch examen behaald. Tijdens de doctoraal fase werd een uitgebreid hoofdvak Organische Chemie (Prof. Dr. B. Zwanenburg) verricht. Een onderzoeksstage Chemische Toxicologie (Prof. Dr. J. Noordhoek) van zes maanden werd verricht in het Research Instituut voor Toxicologie van de Universiteit Utrecht. In het kader van het studentenuitwisselings-programma ERASMUS werd gedurende 6 maanden een extra bijvak Organische Chemie gevolgd aan de Universitá di Bologna, Italie (Prof. Dr. B.F. Bonini). Op 27 mei 1991 werd het doctoraal examen Scheikunde afgelegd.

Van juli 1991 tot juli 1995 was hij als onderzoeker in opleiding, gefinancierd door de stichting Scheikundig Onderzoek Nederland (SON) en de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), verbonden aan de Katholieke Unversiteit Nijmegen. Hier werd het in dit proefschrift beschreven promotie-onderzoek verricht onder leiding van Prof. Dr. B. Zwanenburg in de vakgroep Organische Chemie die deel uitmaakt van de NSR-Onderzoeksschool. In deze periode was hij intensief betrokken bij de begeleiding van studenten voor hun hoofd- en bijvakstages.

Voor de periode november 1996-oktober 1997 is hem op voordracht van Prof. Dr. B. Zwanenburg en Prof. Dr. R.J.M. Nolte een stipendium van de Niels Stensen Stichting toegekend. In deze periode zal hij verbonden zijn als post-doctoral fellow in de groep van Prof. Dr. N.A. Porter aan de Duke University te Durham (North Carolina, USA)

Hij vervulde verschillende functies bij de plaatselijke Tennisvereniging, waaronder voorzitter van de Technische Commissie en afgevaardigde in het Bestuur gedurende de periode 1987-1992.